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Comparative Genome Analysis between Agrostis stolonifera and Members of the Pooideae Subfamily Including Brachypodium distachyon

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COMPARATIVE GENOME ANALYSIS BETWEEN *AGROSTIS STOLONIFERA* AND MEMBERS OF THE POOIDEAE SUBFAMILY, INCLUDING *BRACHYPODIUM DISTACHYON*

A Thesis Presented

by

LORETO ARANEDA

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DEDICATION

A mi familia.

Thanks to God for everything I have experienced.

My parents Manuel and Sara……..all their love and efforts have made me the person I am now.

My brothers and sister, Manolo, Juan Pablo and Paola……..my playmates during my childhood and that make me proud.

My nieces and nephews, Belen, Florencia, Simon and Matias……..they have filled my heart of love.

Carlos and Marjorie……..for being the happiness of my sister and brother.

And my loved husband Roberto the light in my life.

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ABSTRACT

COMPARATIVE GENOME ANALYSIS BETWEEN *AGROSTIS STOLONIFERA* AND MEMBERS OF THE POOIDEAE SUBFAMILY, INCLUDING *BRACHYPODIUM DISTACHYON*

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Understanding of grass genome structure and evolution has been significantly advanced through comparative genomics. The genomes of most cool-season forage and turf grasses, belonging to the Pooideae subfamily of the grasses, remain understudied. Creeping bentgrass (*Agrostis stolonifera*, allotetraploid $2n=4x=28$) is one of the most important cool-season turfgrasses widely used on golf courses due to its low mowing tolerance and aggressive growth habit. Genome analysis of this recently domesticated polyploidy, creeping bentgrass, can add to the understanding of the evolution and domestication of grasses. In this study, an RFLP genetic map of creeping bentgrass using 229 RFLP markers derived from cereal (91) and creeping bentgrass (68) EST-RFLP probes was constructed for a comparative genome analysis. The genetic map of creeping bentgrass (Aveneae tribe) was compared with those of perennial ryegrass (Poeae tribe), oat (Aveneae tribe) and wheat (Triticeae tribe) of the Pooideae subfamily, and rice (Oryzeae tribe) of the Ehrhartoideae subfamily. Large-scale chromosomal rearrangements between the map of creeping bentgrass and the respective maps of the Triticeae, oat, and rice were observed. However, despite their membership in different

tribes of Pooideae, no evidence of chromosomal rearrangements between the maps of creeping bentgrass and perennial ryegrass was detected, suggesting that these recently domesticated species might be closely related than expected. Further comparative genome analysis of creeping bentgrass was performed with whole genome sequences of *Brachypodium distachyon* (Brachypodieae tribe, Pooideae subfamily) using sequences of the above-mentioned RFLP mapped markers and 8,470 publicly available *A. stolonifera* EST (AgEST) sequences. A total of 24 syntenic blocks were identified between the *Agrostis* linkage groups and the *B. distachyon* chromosomes. Orthologous loci of six hundred seventy-eight AgESTs were identified in the *B. distachyon* genome, and these loci can be utilized in further comparative mapping of Pooideae species. Insights from comparative genomics with *B. distachyon* will be useful for genetic improvement of *Agrostis* spp. and provide a better understanding of the evolution of the Pooideae species.

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CHAPTER 1

COMPARATIVE GENOME ANALYSIS BETWEEN *AGROSTIS STOLONIFERA* AND MEMBERS OF THE POOIDEAE SUBFAMILY, INCLUDING *BRACHYPODIUM DISTACHYON*

Introduction

Eukaryotic genomes differ by gene content on chromosomes as well as the order of these genes, which are known as synteny and collinearity, respectively. Angiosperm genomes vary in size and arrangement, even within closely related species. Despite the frequent whole-genome duplication in angiosperms, the number of chromosomes is less variable than genome size. Analyses of changes in genomic structure, such as specific gene rearrangements, insertions or deletions, provide an informative way to clarify relationships among lineages. In the grass family Poaceae, significant variation in DNA content and chromosome number suggest that the evolution of these genomes is fast and dynamic (Tang et al. 2008).

Comparative genomics studies in temperate cereals have been carried out using rice as a model system due to its compact and well-annotated genome sequence. However, the active changes in rice genome sequences, the lack of some biological features, characteristics of a model organism (easy to grow, short plant and life cycle) and the phylogenetic distance from the Pooideae subfamily (that include wheat, barley and temperate forage and turf grasses) lead one to question the utility of this species as a model system. Moreover, rice does not display all the traits that are applicable to study in

temperate grasses, such as fungal resistance, freezing and heat tolerance, wear and injury tolerance and others (Draper et al. 2001; Pacurar, 2009).

A new species, *Brachypodium distachyon* is proposed as a better model system for temperate grasses than rice. *Brachypodium distachyon* is an annual wild grass member of the Brachypodieae tribe and the Pooideae subfamily. Besides its small and complete sequenced genome, *B. distachyon* has additional desirable biological features and a closer phylogenetic relationship to temperate cereals, forage and turf grasses (Draper et al. 2001; Pacurar, 2009; Kumar et al. 2009; Vogel et al. 2010; Garvin et al. 2010).

Comparative genome analysis using a common set of RFLP probes in plants has demonstrated shared gene content and collinearity of genes among related Poaceae species, such as maize, wheat, barley, oat and rice. When whole-genome sequences or physical maps are not available, marker-based mapping is the best approach for a better understanding of the whole-genome (Devos et al. 1993; Van Deynze et al. 1995a, 1995b; Gaut, 2002).

Species of the genus *Agrostis* (known as bentgrasses) are perennial, self-incompatible, and C3 cool-season grasses and are native to the temperate and subarctic climates of Western Europe. The four species commonly accepted as turfgrass types are: 1) creeping bentgrass *A. stolonifera* L. ($2n=4x=28$, A_2 and A_3 genomes), 2) velvet bentgrass *A. canina* L. ($2n=2x=14$, A_1 genome), 3) colonial bentgrass *A. capillaris* L. ($2n=4x=28$, A_1 and A_2 genomes), and 4) redtop bentgrass *A. gigantea* L. ($2n=6x=42$, A_1, A_2, and A_3 genomes). Creeping bentgrass is the most widely used for golf course because of its fine texture, excellent tolerance to low mowing, strong stoloniferous growth habit

and cold tolerance. The genus *Agrostis* is placed in the subfamily Pooideae of the grass family Poaceae that include rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.), oat (*Avena sativa* L.), ryegrass (*Lolium* spp.), and sorghum (*Sorghum bicolor* L.). *Agrostis* is assigned to the tribe Aveneae, which also includes oat (Renvoize and Clayton, 1992; Watson, 1990). Further, the tribes Aveneae and Poeae are closely related (Kellogg, 1998) and in some phylogenies have been combined, but this relationship remains unresolved (Grass Phylogeny Working Group, 2001).

A few studies on molecular marker-based genetic mapping and trait mapping have been conducted in *Agrostis*. The first DNA marker based genetic linkage map of creeping bentgrass (Chakraborty et al. 2005) was developed from a full-sib reference mapping population by crossing two naturalized clones (549 and 372) collected from old golf courses in Wisconsin (Wang et al. 2005). The map covered a distance of 1,110 cM, and contained 14 linkage groups, which were mapped with 424 loci, including expressed sequence tag-restriction fragment length polymorphism (EST-RFLP), randomly amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP). Seven pairs of the homoeologous chromosomes were identified based on duplicated RFLP loci and numbered according to syntenous chromosomes of perennial ryegrass and wheat (Chakraborty et al. 2005). In addition, first genetic linkage map of colonial bentgrass using the backcross population of colonial bentgrass x creeping bentgrass (recurrent parent) mapped with AFLP and gene-based markers was published (Rotter et al. 2009). Recently, QTL mapping of field resistance to dollar spot caused by *Sclerotinia homoeocarpa* was investigated in an enhanced map of the 549 x 372

population using additional EST-RFLP loci (Chakraborty et al. 2006). Comparative genome relationships between *Agrostis* and Poacea spp. need to be established so that genome resources from well-studied species can be utilized to understand genome of underrepresented species for improvement.

One objective of this study was to investigate comparative genome relationships between the EST-RFLP based genetic map of the creeping bentgrass 549 x 372 population and previously published maps of the Triticeae, perennial ryegrass, oat, and rice. For the first time, the creeping bentgrass map is integrated with those members of Poaceae family. A second objective of this study was to explore the utility of *B. distachyon* as a model system for members of Pooideae subfamily. Comparative genome analysis of *A. stolonifera* was performed with whole genome sequences of *B. distachyon* using publicly available sequences of 8,470 *A. stolonifera* EST and sequences of the above-mentioned EST-RFLP mapped markers.

Materials and Methods

Plant material

A two-way pseudo-testcross population, which was developed from the cross between two highly heterozygous creeping bentgrass parental clones 549 and 372, was previously used to develop a genetic map (Chakraborty et al. 2005) using AFLP, RAPD, heterologous cereals and creeping bentgrass EST-RFLP markers. The map density was further enhanced for QTL analysis of dollar spot resistance by mapping additional EST-

RFLP markers (Chakraborty et al. 2006). For comparative genome analysis, an EST-RFLP genetic map was constructed in the current study.

In brief, the controlled crosses were performed in a greenhouse by bagging panicles of both parents with a pollination bag after a field vernalization process. Two parental clones, 549 and 372 were chosen due to differences in morphological traits such as leaf color, shoot density, and root depth, and a significant difference in disease responses to snow mold (caused by *Typhula* spp.) (Wang et al. 2005) and dollar spot (caused by *Sclerotinia homoeocarpa*) (Chakraborty et al. 2006). Six hundred ninety-seven progeny were obtained from seeds harvested from the female clone 549. Of them, a randomly selected progeny of 90 individuals was used for the linkage map construction and for the comparative genome analysis.

RFLP analysis

Young leaves from greenhouse grown plants were harvested, immediately lyophilized and finely ground using a homogenizer. Genomic DNA was extracted from 500 mg of ground tissue using a modified CTAB method (Saghai-Maroof et al. 1984). Ten μg of genomic DNA from each plant was digested with five restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, and *Hind*III) (Promega, Madison, WI). Digested DNAs were loaded onto 1% (w/v) agarose gels and the restriction fragments were separated based on size by electrophoresis for 28 hours at 30V. The DNA from the gels was transferred to Hybond-N+ nylon membranes (Amersham, Piscataway, NJ) by capillary transfer overnight and then treated with optimal crosslink in a UV microwave.

Prehybridization and hybridization were performed in a rotary hybridization chamber (Techne, Inc.) at 65°C. Probes were labeled with ^{32}P by a random priming method (Feinberg and Vogelstein 1983). The membranes were washed in 0.5X SSC and 0.1% SDS for 1 hour at 65°C. Membranes were exposed to X-ray film with intensifying screening for five days at -80°C. The membranes were re-probed 10 to 15 times by first treating them with 0.2M NaOH for 20 min and then with 0.5M Tris/HCL pH 7.5, 0.1X SSC, 0.1% SDS for 20 min at room temperature.

A common set of 152 heterologous anchor probes which contain oat cDNA (CDO), barley cDNA (BCD), and rice cDNA (RZ) from Cornell University, Ithaca, N.Y. (Van Deynze et al. 1998) and additional CDO and BCD probes (the USDA probe depository, Albany, CA) were obtained. Probes with known map locations in the other grass species such as perennial ryegrass, wheat, and oat were initially selected for a further screening step. In addition, 140 creeping bentgrass EST-RFLP probes named "Ast" obtained from two cDNA libraries for each parental clone of the 549 x 372 population (Chakraborty et al. 2005) were first screened against two mapping parents, 549 and 372. Polymorphic probes with a simple and scoreable segregating pattern, showing mostly single or two loci (rarely three or four loci), were selected and tested on the progeny.

Linkage map construction

An EST-RFLP based genetic map was constructed using JoinMap 3.0TM software (Van Ooijen and Voorrips 2001), which can handle out-crossing species using

any marker types with various modes of segregation. For example, analyzed RFLP markers were categorized into one of five segregation types: 1) a heterozygous locus in both parents with four alleles, 2) a heterozygous locus in both parents with one common allele, 3) a heterozygous locus in both parents with two common alleles, 4) a heterozygous locus in the maternal parent, and 5) a heterozygous locus in the paternal parent.

LOD thresholds ranged from 4.0 to 10.0 were used for grouping of markers. The numbering of each pair of homoeologous linkage groups (LGs) was followed according to Chakraborty et al. (2006) and map distances were calculated using the Kosambi mapping function (Kosambi, 1944).

Comparative genome analysis between creeping bentgrass and Triticeae, oat, perennial ryegrass, rice and B. distachyon

The bentgrass 549 x 372 EST-RFLP genetic map was compared with the consensus genetic map of three Triticeae species developed among *T. aestivum*, *T. tauschii*, and *Hordeum* spp. available from the GrainGenes website (http://www.wheat.pw.usda.gov/ggpages/maps.shtml) (Van Deynze et al. 1995c). Comparative map analysis with perennial ryegrass was conducted by comparison with the previously published ryegrass maps (Jones et al. 2002; Sim et al. 2005) and the updated ryegrass map using 13 bentgrass Ast probes (Sim et al. 2007). Comparative map analysis with oat was performed through comparison with the oat map of Van Deynze et al. (1995b). Comparative map analysis with rice was conducted using maps obtained

from Gramene website (http://www.gramene.org/cmap) and with the rice map of Ahn and Tanksley (1993). For the purpose of the comparative genome analysis in this study, a syntenic block between the two species was defined when a segment containing two syntenic loci was not disrupted by a non-syntenic locus or a segment with more than two syntenic loci was not disrupted by two consecutive non-syntenic loci.

Those bentgrass EST-RFLP markers mapped were further analyzed for their putative function by blasting their sequences against protein sequences of known genes at E-value ≤ $1x10^{-5}$ as a cut-off using BLASTX on the NCBI's web server. Map locations of Ast clones were deduced from syntenous chromosome locations in rice using sequence similarity with a *Japonica* rice cDNA clone collection (Kikuchi et al. 2003) and wheat synteny information (Sorrells et al. 2003; http://wheat.pw.usda.gov/pubs/2003/Sorrells). Marker locations and order, detected with the common probes in each respective maps of the Triticeae, ryegrass, oat, and rice, were represented according to the published information cited above.

Because the EST-RFLP markers found in the creeping bentgrass map do not have genetically mapped information in *B. distachyon*, the comparative analysis with this species was performed using BLASTN at *B. distachyon* web site (www.brachybase.org and www.phytozome.net) to determine the chromosomal location, selecting the alignments with E-values ≤ 1×10^{-10}. Moreover, sequences of 8,470 *A. stolonifera* EST (AgEST) publicly available (Rotter et al. 2007) were blasted against the *B. distachyon* genome to find orthologous loci between these two species. Before the BLAST analysis all the redundant AgEST sequences were removed using the CD-HIT-EST program with a sequence identity cut off ≥90% (Li and Godzik, 2006). The identification of orthologs

was based on two more stringent parameters CIP (Cumulative Identity Percentage) and CALP (Cumulative Alignment Length Percentage), and values of CIP ≥60% and CALP ≥70% were used as thresholds (Salse et al. 2008; Kumar et al. 2009).

Results

Linkage map construction

Two hundred-twenty one heterologous RFLP probes (62 BCD, 121 CDO and 38 RZ) were chosen from a common set of 152 anchor probes (Van Deynze et al. 1998) and from additional CDO and BCD probes (the USDA probe depository, Albany, CA) because of their known positions on perennial ryegrass and Triticeae maps (Jones et al. 2002; Sim et al. 2005; Van Deynze 1995a and c). These probes were screened against bentgrass mapping parents and those with strong hybridization signals were selected to test in the progeny (Chakraborty et al. 2005 and 2006). Fifty-five and sixty-two percentages of the barley BCD and oat CDO probes tested, respectively detected simple and scoreable band patterns (data not shown). However, most of the rice RZ probes showed weaker signals than those BCD and CDO probes. Some of the probes with the strong signals were not used due to their complex band patterns with more than two segregating loci per parent, which generated many allelic combinations that made them difficult to score. For example, probe CDO344 generated the most complex band patterns, showing 18 different segregation patterns. In total, 115 probes with simple and clear hybridization signals were tested to determine their segregation among progeny.

Among the tested probes 91 of them were scored and generated 126 markers, which were successfully mapped on 14 LGs (Fig. 1). The other twenty-two probes tested could not be scored because of their faint or smeared bands.

In addition, a total of 140 creeping bentgrass EST-RFLP probes (Ast) were tested for first screening between two mapping parents, 549 and 372. Seventy-two of them exhibited a simple and scoreable segregating pattern, showing mostly single or two loci (rarely three or four loci), were selected and tested on the progeny individuals. Sixty-eight probes generated 103 markers, which were successfully mapped on 14 LGs (Fig. 1).

Five types of RFLP segregation patterns among progenies were detected (ab x cd, ef x eg, hk x hk, lm x ll and nn x np). One locus, heterozygous in one of the parents (lm x ll or nn x np) was the most dominant among the segregation types. The lm x ll type (66%), heterozygous only in the 372 parent, was much more frequent than the nn x np type. Ninety three percent of the mapped markers segregated 1:1 or 1:1:1:1, with a few that segregated 1:2:1. Deviations from the expected segregation ratios (1:1, 1:1:1:1 or 1:2:1) were calculated for all of the mapped markers based on Chi-square contingency tables. Twenty-six percent of the mapped markers deviated significantly from the expected ratios. Particularly on LG5.2, one half of the mapped markers were significantly distorted at $P<0.05$.

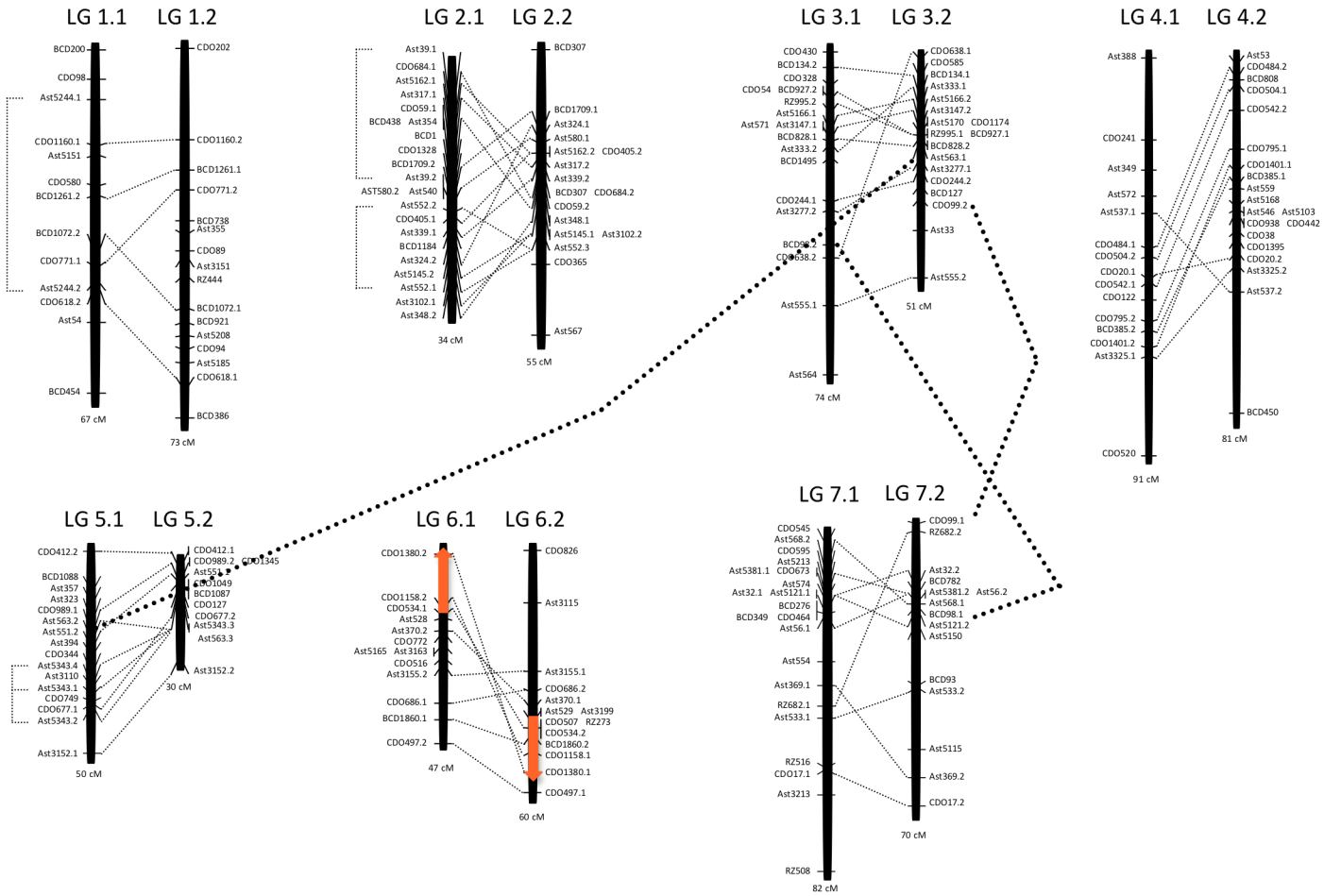


Figure 1. EST-RFLP genetic linkage map of creeping bentgrass.The total genetic length (cM) of each linkage group is indicated below it. The creeping bentgrass, oat, barley and rice cDNAs used as RFLP markers are indicated as Ast, CDO, BCD and RZ followed by the clone number (clone numbers plus '.1', '.2', '.3' or '.4' which show duplicated loci that where linked by a black dotted line). The segment on LGs 6.1 and 6.2, spanning three RFLP markers (CDO1380, CDO1158 and CDO534) and highlighted in orange indicated an inversion and translocation between the two homoeologous LGs.

The final map utilized in the current study included 229 markers generated from 159 RFLP probes including a 42% of those probes that were duplicated (Table 1). The total number of markers was separated into 14 LGs at LOD thresholds ranging from 4.0 to 10.0 (Fig. 1). The numbers for each of the linkage groups (LG1 to LG7) were assigned according to the common probes mapped in genetic maps of the Triticeae (Van Deynze et al. 1995c) and perennial ryegrass (Jones et al. 2002; Sim et al. 2005 and 2007). The numbering of each pair of the homoeologous LGs followed Chakraborty et al. (2005 and 2006). A total of 865 cM was covered in the current genetic map and the genome coverage varied from 30 cM to 91 cM for each LG. The number of mapped marker distribution was from 11 to 22 per LG and the average interval between markers was 3.8 cM in size (Fig. 1).

Comparative genome relationships between creeping bentgrass and Triticeae, oat, perennial ryegrass, rice and B. distachyon

Comparative genome relationships between the map of creeping bentgrass and the respective map of the Triticeae, rice, perennial ryegrass and oat using a common set of heterologous EST-RFLP cereal probes and EST-RFLP creeping bentgrass probes were shown in Figure 2. Overall, comparative mapping showed highly conserved syntenic relationships between creeping bentgrass and the Triticeae, ryegrass, oat and rice.

Besides, the sequences of the Ast probes in the current study were BLAST searched, and 39 of them showed similarity to known genes. Moreover 47 and 32 Ast

probes showed a putative location on rice and wheat chromosomes, respectively (Table 2)

Triticeae

The 53 EST-RFLP loci with known map locations in the Triticeae were evenly distributed on seven pairs of creeping bentgrass LGs (Fig. 2). These markers covered 98% of the bentgrass map (Table 3). Bentgrass LGs 1, 2, 6, and 7 except for a few non-syntenic loci, showed almost complete synteny with corresponding homoeologous chromosomes of the Triticeae. We detected a chromosomal rearrangement on bentgrass LG3.2 relative to Triticeae chromosomes 1 and 3. Bentgrass LG3.2 is represented by the insertion of a segment (covered by Ast5166.2 and Ast5170) of the Triticeae chromosome 1 between two distinct segments of Triticeae chromosome 3. Bentgrass LGs 4.1 and 4.2 contained large-scale chromosomal rearrangements relative to the Triticeae chromosomes 4 and 5. A distal end segment of the Triticeae chromosome 5 flanked by CDO484.1 and CDO504.2 loci on LG4.1 was inserted into the Triticeae chromosome 4. For LG4.2 the same segment flanked by the duplicated loci, CDO484.2 and CDO504.1 was observed. However, due to disruption by a non-syntenic marker (BCD808), a block of synteny could not be established. In addition, bentgrass LG5.1 was represented by segments of Triticeae chromosomes 4 and 5. A segment of Triticeae chromosome 4 flanked by Ast357 and CDO989.1 loci was inserted into Triticeae chromosome 5. In total, we detected three large-scale chromosomal rearrangements in LGs 3, 4, and 5, which can differentiate the bentgrass genome from the Triticeae genome.

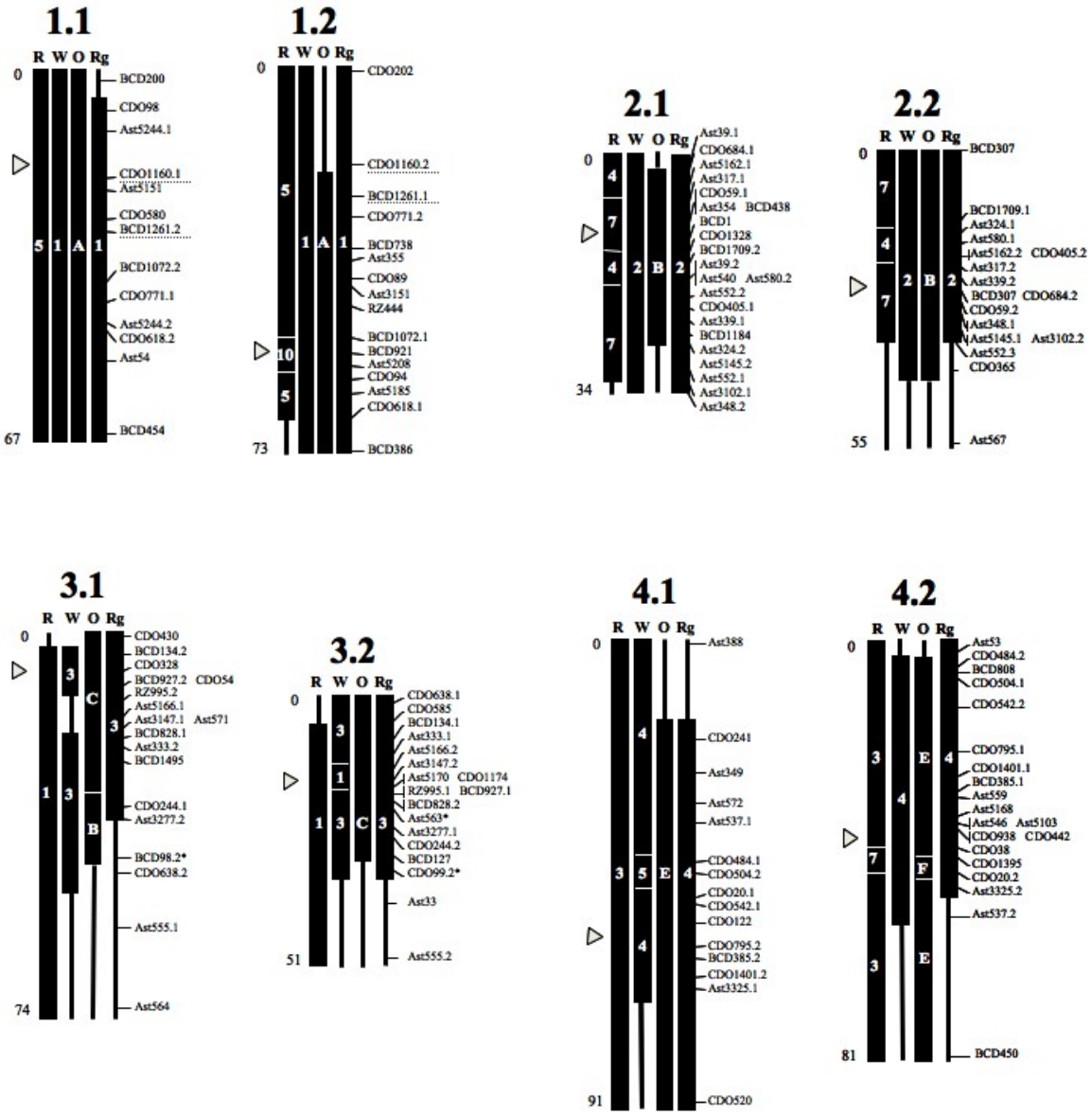


Figure 2. Comparative genome relationships between creeping bentgrass genetic linkage map and the genetic maps of rice (R), Triticeae (W), oat (O) and ryegrass (Rg), respectively, represented for each of the black columns. The markers indicated on the right correspond to those mapped in the creeping bentgrass linkage map (Fig. 1). The number or letter inside the boxes shows the segments of chromosomes or linkage groups from each of the genomes (R, W, O, Rg) that are syntenic to the bentgrass linkage groups. The arrowheads indicate the deduced location of the centromere in bentgrass from the comparisons with Triticeae chromosomes. The total genetic length (cM) of each linkage group is indicated on bottom left.

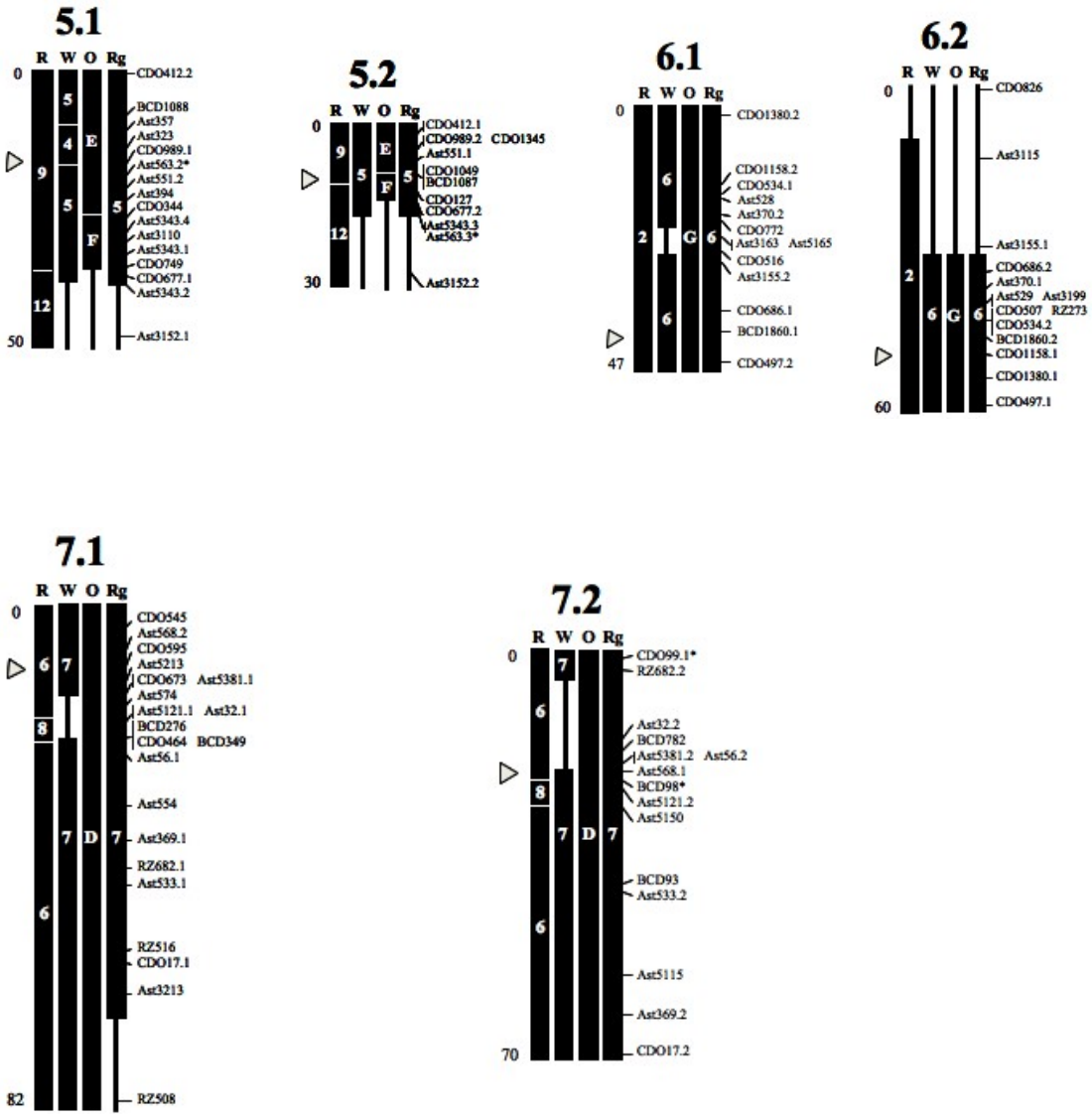


Figure 2. Continued.

Oat

Relatively small coverage of the bentgrass map (76%) was detected by 42 common loci with oat, which were caused by lack of common probes and resulted in a weak syntenic block on bentgrass LG2 (Table 3). Our comparative map indicated that bentgrass LGs 4 and 5 consisted of rearrangements (reciprocal translocation) of oat chromosomes E and F (Fig. 2). Bentgrass LG4.2 was composed of two segments of oat chromosomes E and F. A segment from the most distal area in oat chromosome F between CDO1395 and CDO20.2 loci was inserted between CDO38 and BCD450 loci in oat chromosome E. Bentgrass LGs 5.1 and 5.2 also included two segments of chromosomes E and F. Therefore bentgrass LGs 4 and 5 were differentiated from oat chromosomes E and F. In addition, we detected a large-scale chromosomal rearrangement on bentgrass LG3.1 relative to oat chromosomes B and C. The LG3.1 is represented by the attachment of a segment (flanked by CDO244.1 and BCD98.2 loci) of the oat chromosome B to the distal end of oat chromosome C.

Perennial ryegrass

The 104 common loci were assigned to their locations on the ryegrass map, which covered 92% of the bentgrass map (Table 3). Despite the most number of the common loci shared between the two species, except for some non-syntenic loci, all bentgrass LGs have complete syntenic relationships with those of ryegrass (Fig. 2). Even for bentgrass LGs 4 and 5 which are different from ones of Triticeae and oat, no evidence of large-scale chromosomal rearrangement at the present map resolution was observed between bentgrass and ryegrass.

Rice

The 83 common loci mapped on rice and bentgrass genetic maps covered 82% of the bentgrass map (Table 3). The genome coverage was higher because map locations of the Ast mapped loci were deduced from sequence similarity of bentgrass ESTs with rice. Twenty-nine segments that are derived from all of the rice chromosomes, except for rice chromosome 11, explain all the bentgrass linkage groups (Fig. 2). However, BCD808 on LG4.2 represents rice chromosome 11 and the three duplicated loci generated from Ast563 probe, which were mapped on LGs 5.1, 5.2, and 3.2 represent rice chromosome 11. With the exception of a few non-syntenic loci, both homoeologous pairs of LGs 3 and 6 showed the most conserved syntenic relationships with the homologous chromosomes 1 and 2 of rice, respectively. On the other hands, the other five bentgrass LGs (1, 2, 4, 5, and 7) showed large-scale chromosomal rearrangements relative to rice. Bentgrass LG1.2 was comprised of two syntenic segments from rice chromosomes 5 and 10. A segment containing BCD921 and CDO94 from rice chromosome 10 was inserted into two segments of rice chromosome 5. Only CDO98 on LG1.1 represents rice chromosome 10. Bentgrass LGs 2.1 and 2.2 are represented by segments of rice chromosomes 4 and 7. Bentgrass LG4.2 is represented by the insertion of rice chromosome 7 between two distinct segments of rice chromosome 3. Bentgrass LGs 5.1 and 5.2 are represented by two segments of rice chromosomes 9 and 12. Lastly, LGs 7.1 and 7.2 are represented by the insertion of rice chromosome 8 between two distinct segments of rice chromosome 6.

B. distachyon

Chromosomal location of the EST-RFLP loci mapped in the bentgrass genetic map was deduced by similarity between sequences of the EST-RFLP markers and the *B. distachyon* chromosome sequences (Table 4). One hundred fourteen out of the 160 EST-RFLP markers with significant alignments ($E \leq 1x10^{-10}$) to *B. distachyon* genome were used for analysis of comparative relationship between these two species. Twenty-four segments derived from all the five *B. distachyon* chromosomes were distributed in the 14 bentgrass LGs (Fig. 3). However at the current resolution, only two markers CDO684 and Ast5162 duplicated on LGs 2.1 and 2.2 established a block of synteny with chromosome 5 of *B. distachyon*. Markers CDO1328 on LG2.1, Ast567 on LG2.2, CDO244.1 on LG3.1 and CDO244.2 on LG3.2 also represent chromosome 5 of *B. distachyon*. The most conserved syntenic relationships were found between LG1.1 and chromosome 2, LGs 3.1-3.2 and chromosome 2, LGs 4.1-4.2 and chromosome 1, LG5.2 and chromosome 4, and LGs 6.1-6.2 and chromosome 3 of *B. distachyon*. Out of them, the largest inferred region showing 14 syntenic loci was detected between bentgrass LG4.2 and *B. distachyon* chromosome 1. However, on LGs 3.1 and 3.2 still remain large regions with unknown syntenic relationship to sections of *B. distachyon* chromosomes.

The rest of the bentgrass LGs showed large-scale chromosomal rearrangements (LGs 1.2, 2.1, 2.2, 5.1, 7.1 and 7.2). Bentgrass LG1.2 presents synteny with two segments of *B. distachyon* chromosomes 2 and 3; linkage group 2.1 has two blocks of synteny with chromosomes 1 and 5.

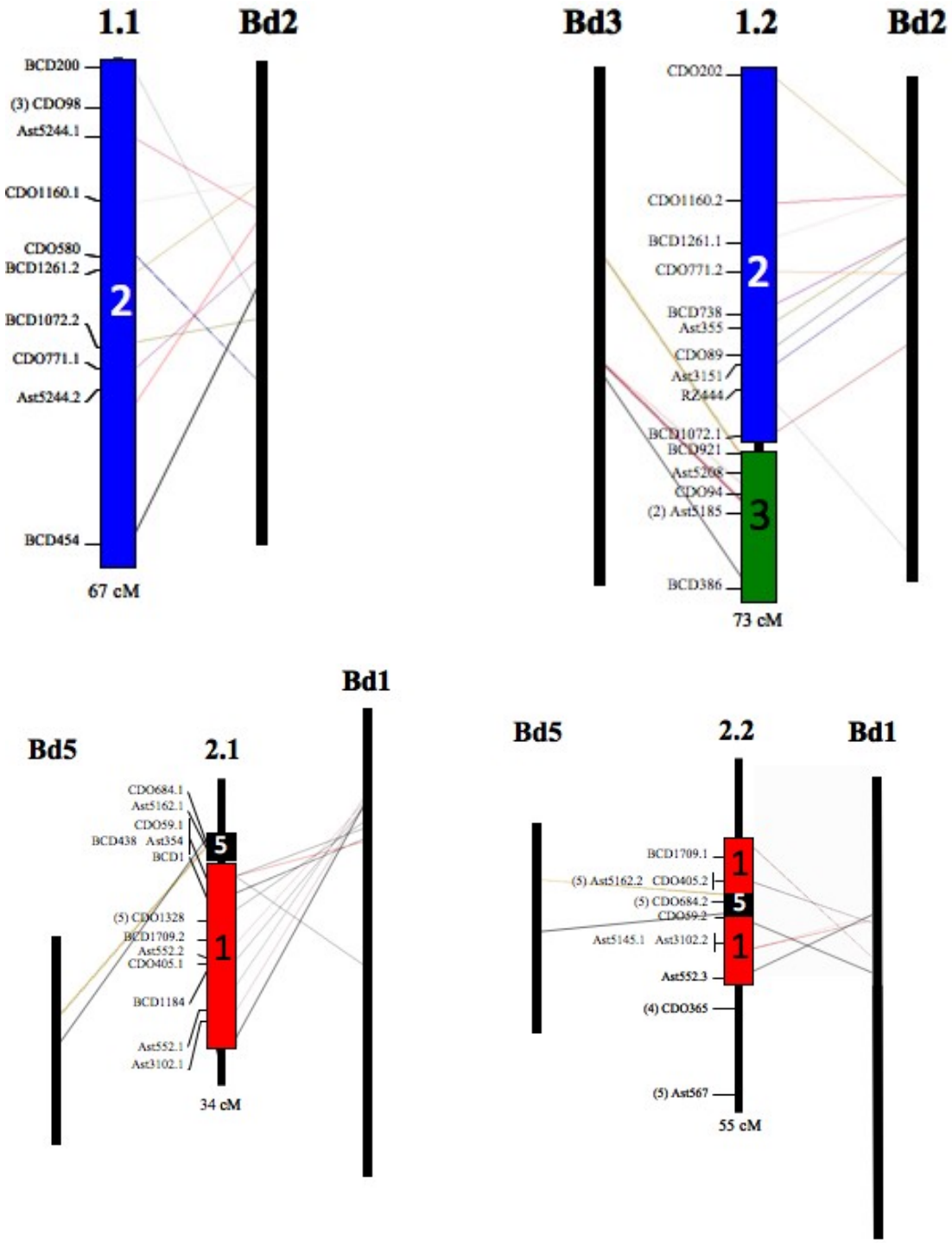
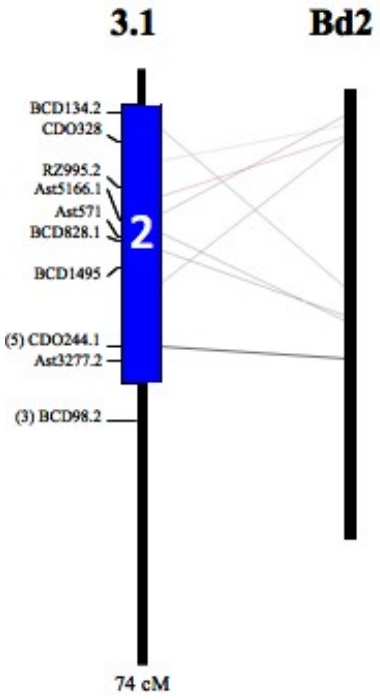
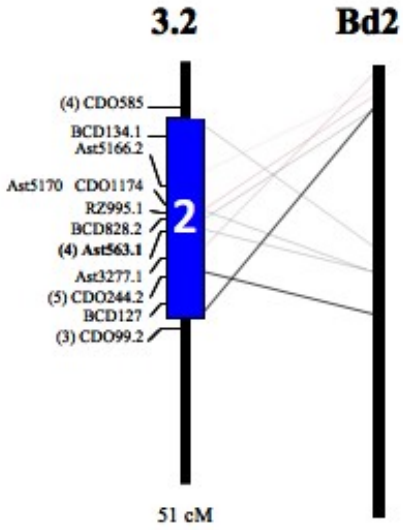
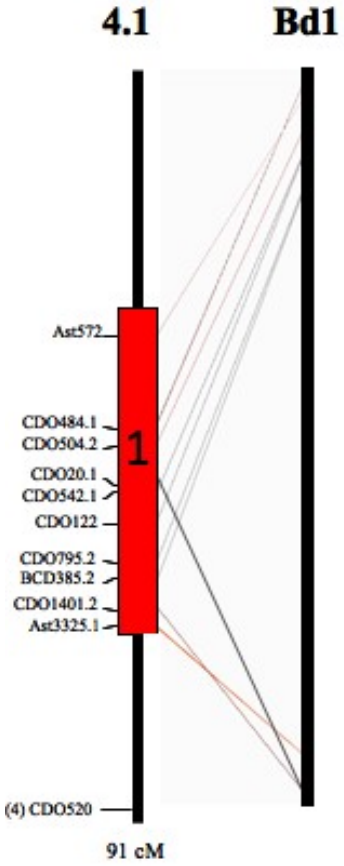


Figure 3. Comparative genome relationship between creeping bentgrass and Brachypodium distachyon. Each of the bars represents the bentgrass linkage groups (total length in cM, below the bar) as showed in Fig.1. The boxes correspond to the conserved synteny to a segment of Brachypodium chromosome (number inside). The position of nonsyntenic markers within a block of synteny is showed in parenthesis next to the marker name. Markers in bold, within a block of synteny, were additionally located in a different chromosome (indicated in parenthesis next to the marker). The collinearity is represented by the color lines that link the markers with the highly similar sequences located in *B. distachyon* chromosomes (Bd)







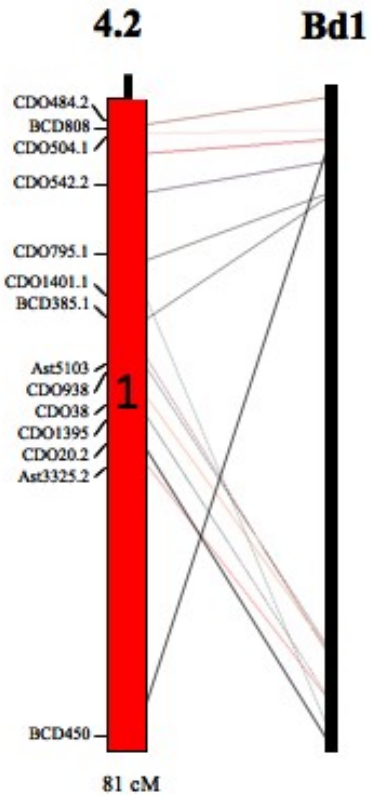


Figure 3. Continued.

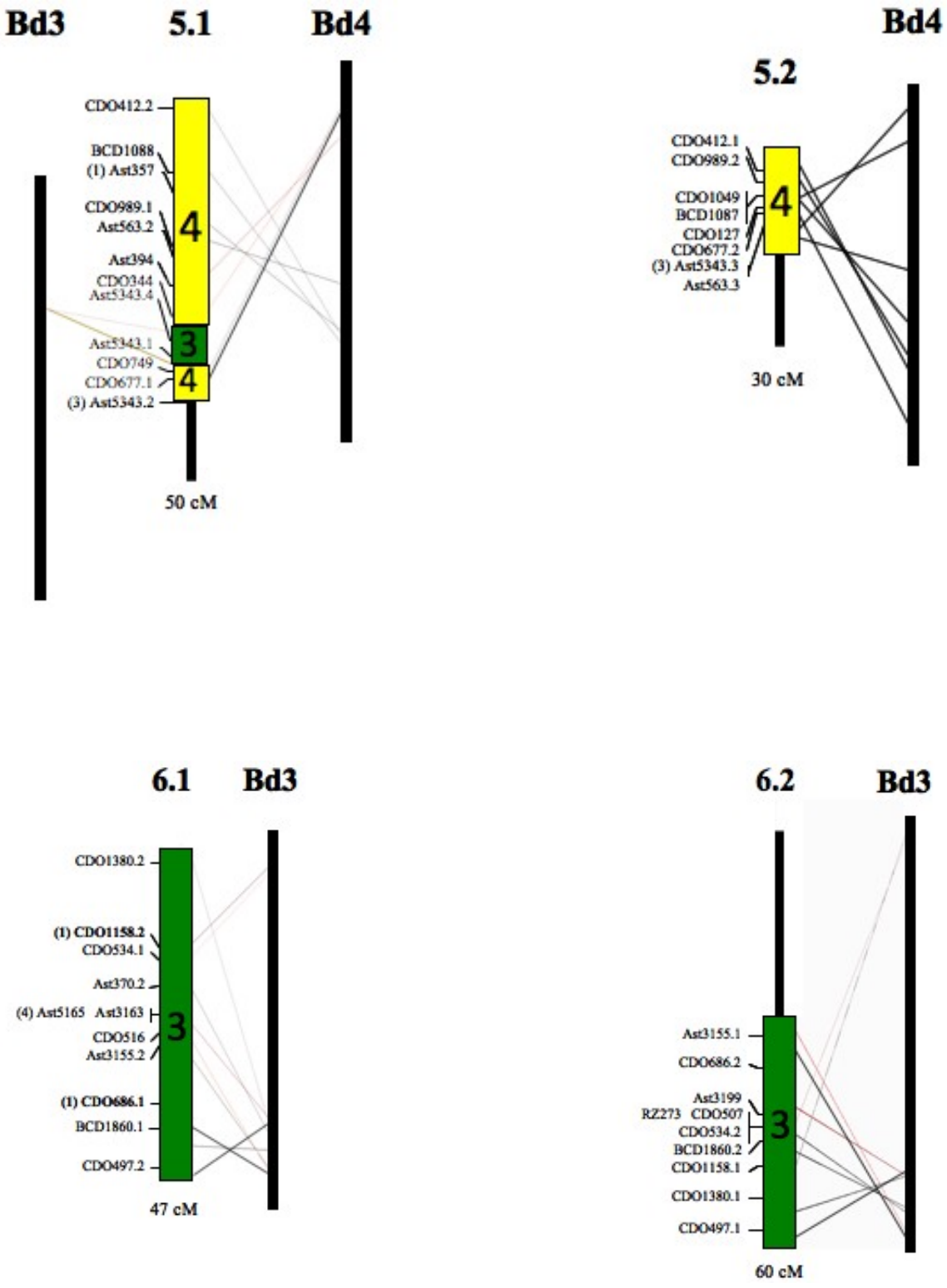


Figure 3. Continued.

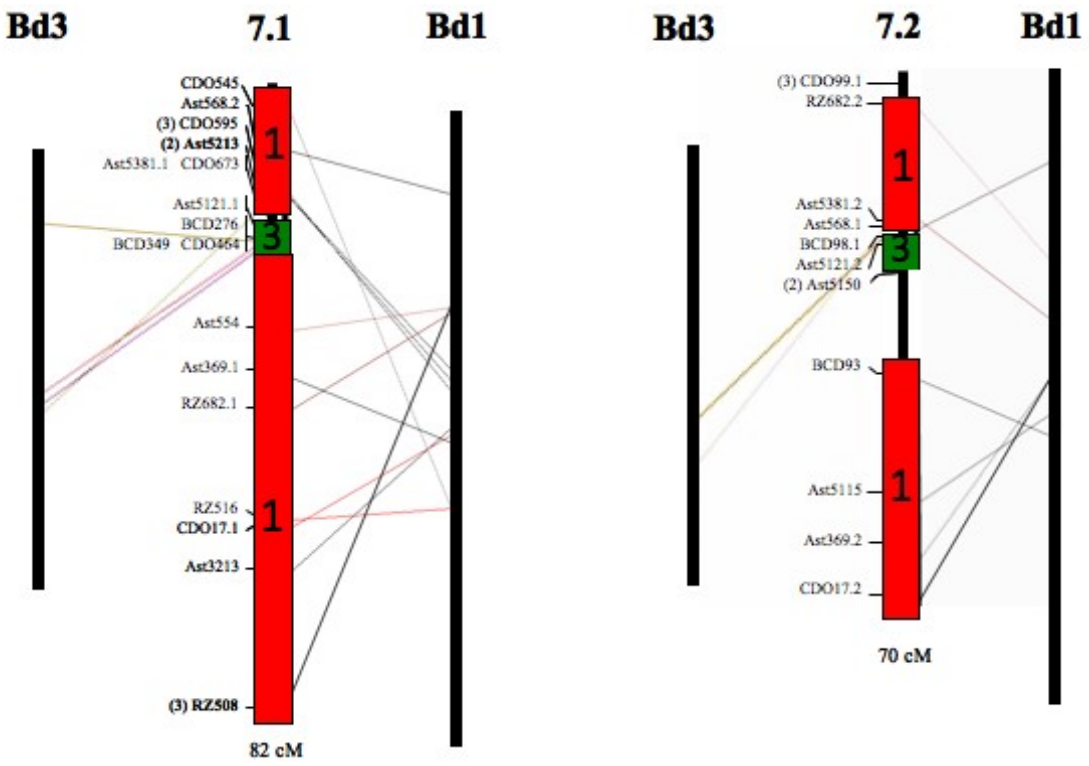


Figure 3. Continued.

Linkage group 5.1 is represented by the insertion of a segment of chromosome 3 between two segments of chromosome 4, a similar rearrangement in LGs 7.1 and 7.2 but in these cases the insertion of chromosome 3 is between two segments of *B. distachyon* chromosome 1.

Linkage group 2.2 did not show chromosomal rearrangement but only showed four loci in a block syntenic to B. distachyon chromosome 1 (Fig. 3). However the rest of the loci (6) were found either in chromosomes 1, 4 or 5 and this could represent a possibility of additional insertions of one or all these B. distachyon chromosomes.

The orthology analysis between 8,470 *A. stolonifera* ESTs (AgEST) and *B. distachyon* genome showed 678 non-redundant AgEST orthologs to *B. distachyon* [196 AgEST in Ch1 (29%), 136 AgEST in Ch2 (20%), 151 AgEST in Ch3 (22%), 104 AgEST

in Ch4 (15%) and 91 AgEST in Ch5 (14%)] in Figure 4. Ninety five percent of these orthologous loci corresponded to unique loci and the rest were found duplicated among and within *B. distachyon* chromosomes (Table 5).

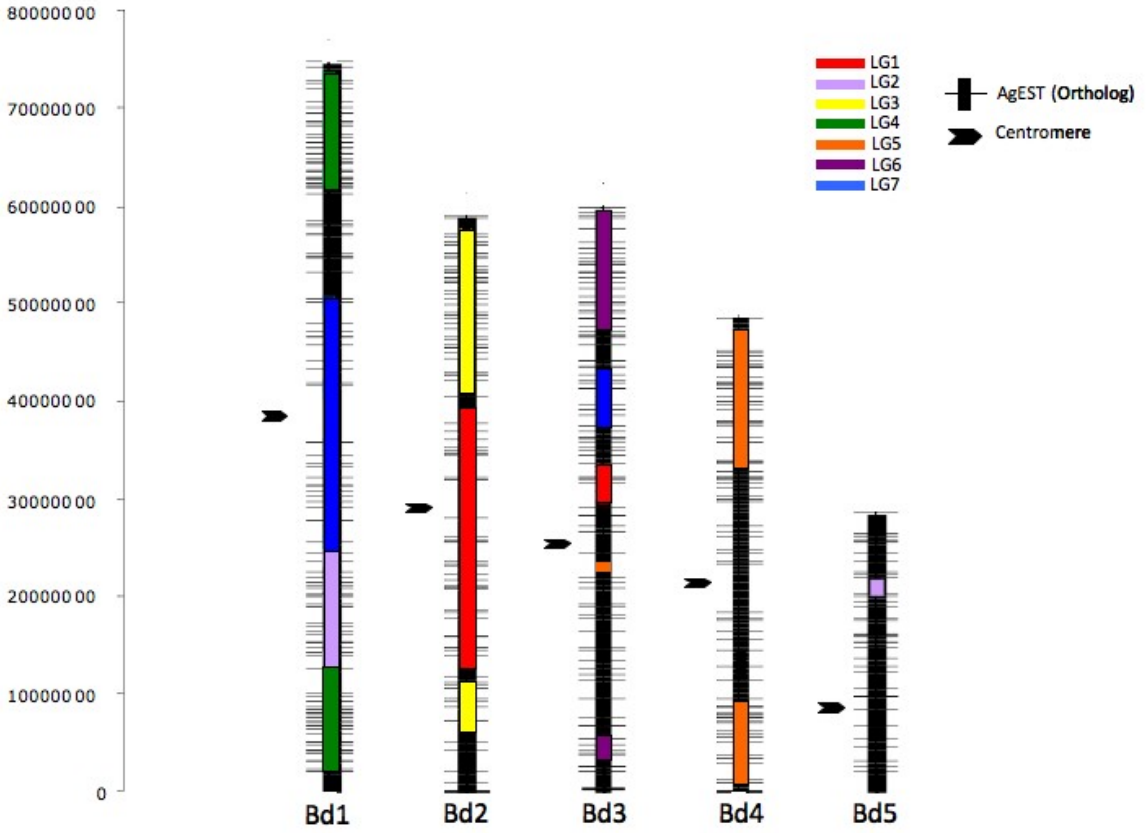


Figure 4. Creeping bentgrass ESTs orthologous to *Brachypodium distachyon* and their location in the physical map of *B. distachyon* (in base pairs). Bd1, Bd2, Bd3, Bd4 and Bd5 represent the *B. distachyon* chromosomes (1 to 5) and horizontal lines for the position of the 678 orthologs. The segments of different colors indicate the creeping bentgrass linkage groups that have synteny with a specific region of *B. distachyon* chromosomes. The arrowheads indicate the centromeric region.

Discussion

Creeping bentgrass EST-RFLP map

The total RFLP-based map size is 865 cM, which is shorter than a previously published creeping bentgrass genetic map (1,110 cM in Chakraborty et al. 2005 and 2006). This difference may be due to the number and type of markers mapped (229). Other marker systems such as AFLP may make the linkage map length longer due to double crossing-overs. For example, AFLP maps in perennial ryegrass have the longest length (Bert et al. 1999). Genotyping errors should also be considered.

In this study, sixty-eight Ast probes generated 103 RFLP markers, which were mapped on 14 LGs (Fig. 1). The sequences of those Ast probes showed similarity to known genes and a putative location on rice and wheat chromosomes (Table 3). These results reflect that Ast probes have the high potential for use in genomic analysis not only for creeping bentgrass but also other for bentgrass spp.

Two subgenomes of creeping bentgrass

Agrostis species are suggested to have some of the most complex genome structures among other genera within the grass family (Bonos et al. 2002). Early cytological studies performed by Jones (1956a, b, and c), studies of disomic inheritance of polymorphic isozymes, the published RFLP based linkage map with duplicated markers likely originated from the two subgenomes (A_2 and A_3) and the ratio of coupling

vs. repulsion linkages between markers, suggest that creeping bentgrass is a strict allotetraploid comprised of two A_2A_2 and A_3A_3 subgenomes (Warnke et al. 1998; Chakraborty et al. 2005 and 2006). At the current map resolution, a high percentage (42%) of the mapped RFLP markers were duplicated and evenly distributed on the seven pairs of homoeologous LGs (Fig. 1) which further confirmed an allotetraploid origin suggested by Jones (1956a, b, and c), Warnke et al. (1998), and Chakraborty et al. (2005). Duplication of markers within and among LGs in Figure 1 were detected in following probes: Ast5244, Ast39, Ast5343, Ast552, Ast563, BCD98 and CDO99. Evidence of inversion and translocation between distal end segments of two homoeologous LGs 6.1 and 6.2 indicated that two subgenomes of creeping bentgrass could have gone through major chromosomal rearrangements. However, more research is required to confirm whether duplications of markers are within homologous chromosomes before homoeologous chromosomes are differentiated. Unfortunately, diploid bentgrass species with either A_2 or A_3 genome are presently unknown. Colonial and velvet bentgrasses have been proposed to have genome compositions $A_1A_1A_2A_2$ and A_1A_1 based on cytological evidence, respectively, where the A_1A_1 genome is common. Recent DNA sequences based phylogenic analysis suggested that genome designation for velvet bentgrass should be A_2 genome (Rotter et al. 2010). Comparison of these two species' maps mapped with the same set of RFLP probes uniquely present in each genome should reveal the chromosomes of the A_2 genome common in creeping and colonial bentgrasses.

Synteny between creeping bentgrass and Triticeae, ryegrass, oat, rice and B. distachyon, and genome level affinity

A high level of conserved synteny between creeping bentgrass and other grass species such as Triticeae, ryegrass, oat and rice were observed in the current study. Comparative mapping studies within Poaceae species also revealed evidence of unique chromosomal rearrangements. Kellogg (1998) studied genome structure within the grass family using comparative mapping and suggested that all species belonging to the Pooideae subfamily have two unique chromosomal rearrangements (5-10-5 and 6-8-6) relative to rice. As a member of the Pooideae subfamily, creeping bentgrass in the current study clearly shows the 5-10-5 and the 6-8-6 rice rearrangements on creeping bentgrass LGs 1 and 7, respectively as observed in ryegrass (Sim et al. 2005).

The bentgrass LGs fell in the 12 groups of orthology between rice and wheat defined by Salse et al. (2008), however LGs 4 and 5 show differences due to lack of synteny with chromosome 11 of rice. When we compare creeping bentgrass with rice at this level of map resolution, only four markers (CDO365, CDO520, BCD808 and Ast563) represent this rice chromosome in six different bentgrass LGs (2.2, 3.2, 4.1, 4.2, 5.1, and 5.2). The poor synteny with this rice chromosome may be attributable to the high fraction of repetitive sequences described in rice chromosomes 11 and 12 (29.5% and 31.6%, respectively) producing a disruption of the synteny (The Rice Chromosomes 11 and 12 Sequencing Consortia, 2005).

Based on comparisons of morphological characters, bentgrass and oat belong to the same Aveneae tribe, while ryegrass belongs to the Poeae tribe (Renvoize and Clayton,

1992). Results of our comparative genome analyses show strong syntenic relationship between bentgrass and ryegrass, with only four non-syntenic markers. On the other hand, events of large-scale chromosomal rearrangements relative to oat were detected on bentgrass LGs 3, 4 and 5. Results suggest that bentgrass is more closely related to ryegrass than to oat in terms of genome structure, despite a closer taxonomic affinity between bentgrass and oat. These results combined with previous results in the recently domesticated species ryegrass provide strong evidence to dispute the currently accepted taxonomic relationship among species within Pooideae subfamily, which Jones et al. (2002) and Sim et al. (2005) first brought into question. Additionally, Rotter et al. (2007) carried out the first and only phylogenetic analysis that included two members of *Agrostis* genus (*A. stolonifera* "creeping bentgrass" and *A. capillaris* "colonial bentgrass") based on sequences of conserved ortholog sets (COS). This study clustered creeping and colonial bentgrasses more closely to tall fescue (tribe Poeae) than to oat (tribe Aveneae). Therefore, these recently domesticated species, bentgrass and ryegrass, might be closely related and "wild" grass species of the Poaceae family.

Based on the common sets of markers, when creeping bentgrass is compared to rice, the number of chromosomal rearrangements is eight, on the LG1.2, 2.1-2.2, 4.2, 5.1-5.2 and 7.1-7.2. The same types of rearrangements were previously reported in ryegrass when it is compared to rice (Sim et al., 2005). However when creeping bentgrass is compared to *B. distachyon*, this number is reduced to six because LGs 4.1 and 4.2 do not show any evidence of chromosomal rearrangements. Nevertheless we observed many changes in the order of markers within blocks of synteny across the different LGs (Fig.3), a situation that could indicate that gene movements within chromosome have taken place

with a relatively high frequency (de la Rota, 2004). Despite all the *B. distachyon* chromosomes are represented across the 14 LGs of creeping bentgrass, chromosome 5 of *B. distachyon* is significantly underrepresented (only two syntenic blocks defined by two markers, Fig. 3). According to Vogel et al. (2010) the high LTR retrotransposon density found in this *B. distachyon* chromosome could increase syntenic disruptions.

We believe that one way to establish a more accurate syntenic relationship with *B. distachyon* chromosomes is to develop molecular markers from the creeping bentgrass EST orthologous to the *B. distachyon* genome identified in this study, which could serve as a roadmap in order to enhance the *Agrostis* linkage map. As a preliminary attempt we have designed intron-spanning primers (data not shown) based on *B. distachyon* genome sequences and creeping bentgrass EST orthologs (Feltus et al. 2006). These primers were screened in nine species from the Aveneae, Poeae and Brachypodieae tribes and around 70% of them amplified strong polymorphic bands in all the species. Future linkage studies could be performed using intron-spanning markers, considering the close proximity of introns to exons and the conserved position of introns.

A previous study detected the most robust QTL for dollar spot resistance on LG7.1 of the creeping bentgrass linkage map (Chakraborty et al. 2006). The same authors mention that QTL for SHB-TRS (sheath blight resistance caused by *Rhizoctonia solani*) could be detected close to the dollar spot resistance QTL, since both fungi may have similar pathogenicity. Based on our comparative mapping, there is a highly similar genome structure between creeping and ryegrass. Previous studies have located QTL for crown rust and powdery mildew resistance in linkage group 7 of ryegrass genetic map (Sim et al. 2007 and Schejbel et al. 2008). With this limited information we could suggest

that LG7.1 of creeping bentgrass may has a more important role in disease resistance than the rest of the linkage groups. When we compare creeping bentgrass with rice for syntenic QTL information, we found that the QTL for SHB-TRS resistance is located on chromosome 11 of rice (Channamallikarjuna et al. 2010) and several genes of agronomic importance (blast, bacterial, virus, insect resistance and others) have been mapped in rice chromosomes 11 and 12, moreover the resistance and defense response genes [leucine-rich repeat (LRR), protein kinase and NB-ARC domains] are enriched on these two chromosomes relative to the whole genome of rice (The Rice Chromosomes 11 and 12 Sequencing Consortia, 2005). However due to lack of synteny with this rice chromosome, it is not possible to establish a link between the genetic information of rice and the QTL information of creeping bentgrass at an equivalent level of resolution.

In addition to this, when we examined the putative function of the complete data set of creeping bentgrass EST used in this study we found 13 of them showed similarity to genes associated with defense or disease resistance. The bentgrass ESTs with hits from the *B. distachyon* proteome and homologous genes were localized mainly to *B. distachyon* chromosomes 1, 2 and 3 (Table 6). As shown in Figure 3, chromosomes 1 and 3 of *B. distachyon* show synteny with bentgrass LG7.1 where the major QTL for field resistance to dollar spot was found. Additionally, Chakraborty et al. (2006) detected QTL with smaller effect in LGs 7.2, 2.1, 3.2, 4.1, 4.2 and 6.2 that present synteny with *B. distachyon* chromosomes 1, 2 and 3. A recent study indicated a higher level of synteny and conservation of resistance gene positions between *B. distachyon* and barley, as compared with rice and barley (Drader and Kleinhofs, 2010). Although rice has been extensively used as a model system for grasses, genome information of *B. distachyon*

could be easily transferable for comparative studies, at least in Pooideae spp. due to evolutionary closeness. Future studies should explore the relationship between QTL of traits of interest in the bentgrass linkage map and genes located in syntenic areas of *B. distachyon*.

In conclusion, the current genetic map of allotetraploid creeping bentgrass was, for the first time, used for comparative genome analysis with cereals based on a common set of cereal and creeping bentgrass EST-RFLP probes. This comparative map establishes a first look in elucidating genome organization among bentgrass species as well as understanding the genome relationships of bentgrass with Triticeae, ryegrass, oat, and rice. Evidence of large-scale chromosomal rearrangements, which differentiate the bentgrass genome from genomes of Triticeae, oat, and rice, were observed based on syntenic markers, and need further validation. However, no evidence of large-scale chromosomal rearrangement was detected between two recently domesticated species, bentgrass and ryegrass suggesting that these two species might be more closely related than expected.

Our future goal is to utilize genetic information from well-studied model species like rice and now *B. distachyon* through comparative mapping, to locate ortholog genes of traits of interest in bentgrass and eventually to develop improved creeping bentgrass cultivars using classical and molecular breeding approaches. Currently, this map has served to detect a QTL for field resistance to dollar spot (Chakraborty et al. 2006) and will be utilized for mapping QTLs and functional markers for important traits of interest in the golf industry such as drought, cold, heat, salt and disease tolerance, as well as leaf color, aggressiveness, and shoot density.

APPENDIX

TABLES

Table 1. Duplicated RFLP loci derived from a common set of heterologous cereal and creeping bentgrass EST-RFLP probes that were mapped in the 549 x 372 mapping population of creeping bentgrass.

	No. of probes generating multiple loci				
Source of probe [a]	1 locus	2 loci	3 loci	4 loci	Total
Ast	37	28	2	1	68
BCD	20	9	0	0	29
CDO	32	24	0	0	56
RZ	4	2	0	0	6
Total	93	63	2	1	159

[a] Ast, BCD, CDO, and RZ probes derived from creeping bentgrass, barley, oat, and rice cDNAs, respectively.

Table 2. Creeping bentgrass cDNA clones mapped on 14 linkage groups of the creeping bentgrass reference population (549 x 372) (Chakraborty et al. 2005 and 2006). The *e*-value of the cDNA sequence that matches to database (BLASTX) and the map position of the bentgrass ESTs relative to rice (Kikuchi et al. 2003) and wheat (Sorrells et al. 2003; http://wheat.pw.usda.gov/pubs/2003/Sorrells/) are indicated.

Bentgrass cDNA clone	Putative function	*e*-value	Putative location on chromosomes		Bentgrass LG
			Wheat	Rice	
Ast5151	putative U2 snRNP auxiliary factor [*Oryza sativa*]	7e-08	4	5	1
Ast54	No significant similarity found.	-	1	5	1
Ast355	phosphoglycerate kinase, putative [*Arabidopsis thaliana*]	6e-09	1	5	1
Ast3151	unknown protein [*A. thaliana*]	1e-40	1	5	1
Ast5208	glycine cleavage system H protein, mitochondrial, putative [*A. thaliana*]	2e-63	1	10	1
Ast39	chitinase (EC 3.2.1.14) precursor [*Beta vulgaris*]	2e-06	2	4	2
Ast348	No strong matches	-	-	-	2
Ast3102	Mg-chelatase subunit XANTHA-F [*Hordeum vulgare*]	2e-53	2	3	2
Ast540	homeobox-leucine zipper protein homolog [*Glycine max*]	2e-14	-	4	2
Ast5145	ribosomal protein L12.1 precursor, chloroplast [*Secale cereale*]	2e-53	-	7	2
Ast580	No hit	-	2	4	2
Ast567	thylakoid lumen 15.0 kDa protein [*A. thaliana*]	2e-39	-	7	2
Ast5162	chlorophyll A-B binding protein, chloroplast (LHCB6) [*A. thaliana*]	1e-47	2	4	2
Ast33	No hit	-	-	1	3
Ast3277	putative histidine kinase [*O. sativa*]	1e-17	3	1	3
Ast555	oxygen-evolving enhancer protein 3-1, chloroplast precursor (16 kDa subunit photosystem II) [*O. sativa*]	1e-45	-	1	3
Ast564	No hit	-	-	1	3
Ast571	oat victorin binding protein [*Avena sativa*]	5e-64	3	1	3
Ast5166	metallothionein-like protein type 3 [*H. vulgare*]	6e-14	1	1	3

Ast5170	Aux/IAA protein [*Solanum tuberosum*]	1e-34	1	1	3
Ast388	No hit	-	4	3	4
Ast349	unknown protein [*O. sativa*]	2e-11	4	3	4
Ast572	putative methylenetetrahydrofolate reductase [*O. sativa*]	e-117	-	3	4
Ast546	putative serine/threonine-specific protein kinase [*O. sativa*]	3e-62	4	3	4
Ast5103	putative serine/threonine kinase [*O. sativa*]	1e-43	4	3	4
Ast5168	No hit	-	-	6	4
Ast559	nonspecific lipid transfer protein Cw-18 precursor - barley [*H. vulgare*]	6e-27	2	12	4
Ast53	Serine hydroxymethyltransferase, mitoch. Precursor [*S. tuberosum*]	0.0	4	3	4
Ast357	PS I 10.8 kDa reaction center subunit IV, chloroplast precursor (PSI-E) [*H. vulgare*]	6e-21	4	7	5
Ast323	No hit	-	-	9	5
Ast3110	No hit	-	3	4	5
Ast563	fructose-bisphosphate aldolase (EC 4.1.2.13) [*O. sativa*]	4e-47	5	11	5
Ast3163	expressed protein [*A. thaliana*]	3e-59	1	2	6
Ast3115	putative brown planthopper susceptibility protein Hd002A [*O. sativa*]	2e-34	-	2	6
Ast3199	chloroplast translational elongation factor Tu [*O. sativa*]	e-115	6	2	6
Ast528	putative WRKY9-10 protein [*H. vulgare*]	4e-05	6	2	6
Ast529	phosphoribulokinase; ribulose-5-phosphate kinase [*T. aestivum*]	2e-57	7	2	6
Ast32	putative auxin-regulated protein [*O. sativa*]	2e-54	1	8	7
Ast369	ALDH2b mRNA for mitochondrial aldehyde dehydrogenase [*Secale cereale*]	0.0	-	-	7
Ast3213	chlorophyll a/b binding protein precursor [*H. vulgare*]	2e-97	-	6	7
Ast56	putative UOS1 [*O. sativa*] (Pred. nucleoside-diphosphate-sugar epimerases: weaker)	5e-96	7	6	7
Ast533	knotted 6 [*H. vulgare*] / Putative homeobox gene [*O. sativa*]	3e-60	7	6	7
Ast554	vacuolar proton-inorganic pyrophosphatase [*H. brevisubulatum*]	3e-58	7	6	7

Ast5381	Ras-related GTP-binding family protein [*A. thaliana*]	4e-50	-	6	7
Ast5213	putative cytochrome B5 [*O. sativa*]; susceptibility homeodomain transcription factor [*O. sativa*]	1e-43	3	6	7
Ast568	putative MATE efflux family protein [*O. sativa*]	3e-82	3	3	7
Ast574	No hit	-	-	6	7
Ast5150	nucleosidase-related [*A. thaliana*]	5e-22	-	1	7
Ast5115	putative protein kinase [*O. sativa*]	1e-23	-	6	7

Table 3. Genome comparisons of the 549 x 372 map of *Agrostis stolonifera* with previously published maps of *Triticum*, *Lolium*, *Avena*, and *Oryza sativa* based on a common set of heterologous cereal and creeping bentgrass EST-RFLP probes.

Species	Number of common loci	Number of conserved syntenic block	Number of chromosomal rearrangements	Genome coverage (%) [a]
Triticum [b]	53	24	3	98
Lolium [c]	104	14	0	92
Avena [d]	42	19	4	76
Oryza sativa	83	29	8	82

[a] Genome coverage in bentgrass (%) = (a/b)x100, where a = total map length (cM) represented in bentgrass with probes mapped in each species (Triticeae, ryegrass, oat, or rice), b = total map length (cM) of bentgrass.
[b] Triticeae tribe consensus maps for *T. aestivum*, *T. tauschii*, and *Hordeum* spp.
[c] *Lolium* map was derived from diploid MFA x MFB interspecific cross between *L. multiflorum* Lam. and *L. perenne* L.
[d] *Avena* map was derived from diploid interspecific cross between *A. atlantica* and *A. hirtula*

Table 4. *B. distachyon* chromosome location of sequences of EST-RFLP markers mapped on genetic map of creeping bentgrass.

Bentgrass LG[a]	Marker (EST ID)[b]	Bd chromosome[c]	Bd chromosome location[d]	
			Start	End
1.2	CDO202	2	13312741	13313054
1.1-1.2	BCD1261	2	13918752	13919099
1.1-1.2	CDO1160	2	13959438	13959911
1.1	Ast5244 (DY543632)	2	17734568	17737309
1.2	BCD738	2	18485474	18485955
1.2	Ast355 (DY543397)	2	18485698	18485954
1.2	CDO89	2	19921391	19922005
1.2	Ast3151[e]	2	22117132	22119285
1.1-1.2	CDO771	2	22596648	22597674
1.1	BCD454	2	23921191	23921611
1.1-1.2	BCD1072	2	30224103	30224321
1.1	BCD200	2	30230242	30230609
1.2	Ast5185 (DY543548)	2	36219723	36221116
1.1	CDO580	2	38466405	38467009
1.2	RZ444	2	53296932	53297140
1.2	BCD921	3	20772298	20772621
1.1	CDO98	3	30319151	30321086
1.2	BCD386	3	32086894	32087540
1.2	Ast5208 (DY543591)	3	32651725	32653364
1.2	CDO94	3	32721646	32722742
2.1	BCD1184	1	13478584	13478963
2.1-2.2	Ast552 (DY543414)	1	14595910	14600036
2.1-2.2	Ast5145 (DY543455)	1	14908782	14909109
2.1-2.2	Ast3102[e]	1	15401288	15401827
2.1-2.2	CDO405	1	15743376	15743751
2.1-2.2	BCD1709	1	19259600	19259999
2.1-2.2	CDO59	1	20953161	20953582
2.1	BCD1	1	23483880	23484122
2.1	Ast354 (DY543396)	1	24105397	24107492
2.1	BCD438	1	53957117	53957391
2.1	BCD1184	1	64356335	64357924
2.2	CDO365	4	17638266	17638709
2.2	Ast567 (DY543447)	5	4337487	4339261
2.1-2.2	Ast5162 (DY543463)	5	15617190	15617775
2.1	CDO1328	5	15844066	15847157
2.1-2.2	CDO684	5	21100968	21101188
3.2	**Ast563** (DY543444)	2	841745	842266
3.1-3.2	Ast5166 (DY543467)	2	4664467	4665522
3.2	Ast5170 (DY543421)	2	6119943	6123643
3.1	CDO328	2	6867698	6868202
3.2	BCD127	2	8725766	8726125
3.1-3.2	RZ995	2	9220956	9221418

3.1	BCD1495	2	9496671	9497017
3.1	BCD1495	2	10491102	10491445
3.1	BCD1495	2	10495830	10496174
3.1-3.2	BCD134	2	41622340	41623472
3.1-3.2	BCD828	2	47227872	47228154
3.2	CDO1174	2	47437527	47437914
3.1	Ast571 (DY543450)	2	48360330	48361363
3.1-3.2	Ast3277 (DY543641)	2	56909915	56911116
3.1-3.2	Ast3277 (DY543641)	2	58867188	58867992
3.1-3.2	Ast3277 (DY543641)	2	58868700	58869561
3.1	BCD98	3	44128540	44129179
3.2	CDO99	3	44130090	44130609
3.2	CDO585	4	7093558	7093796
3.2	**Ast563** (DY543444)	4	29522820	29523351
3.1-3.2	CDO244	5	24490389	24490639
4.1-4.2	CDO484	1	1366964	1367495
4.1	Ast572 (DY543451)	1	2811156	2813423
4.1	Ast349 (DY543394)	1	3103523	3104213
4.2	BCD808	1	4998784	4999139
4.1-4.2	CDO504	1	5962259	5962873
4.2	BCD450	1	5988342	5988796
4.2	BCD808	1	7318997	7319438
4.1-4.2	CDO542	1	8350647	8351378
4.1	CDO122	1	8567153	8567563
4.1-4.2	CDO795	1	11769377	11770588
4.1-4.2	BCD385	1	12074984	12075099
4.2	BCD808	1	19135955	19136422
4.2	CDO938	1	62486394	62487017
4.2	CDO38	1	62730415	62730828
4.2	Ast5103 (DY543488)	1	63213682	63215164
4.2	CDO1395	1	68017621	68018050
4.1-4.2	Ast3325 (DY543730)	1	68060410	68061124
4.1-4.2	CDO1401	1	71738939	71739260
4.1-4.2	CDO20	1	72945681	72946010
4.1-4.2	CDO20	1	72951260	72951645
4.1	CDO241	4	11555301	11555602
4.1	CDO520	4	14398184	14398326
4.2	Ast559 (DY543419)	4	31093780	31094131
5.1	Ast357 (DY543398)	1	24421682	24422146
5.2	**CDO1049**	3	19476497	19477936
5.1-5.2	Ast5343 (DY543747)	3	21176323	21178461
5.1-5.2	Ast551 (DY543413)	3	21620907	21621429
5.1	CDO749	4	1558290	1559232
5.1-5.2	CDO677	4	2023698	2024300
5.1	CDO344	4	3112832	3113225
5.1	Ast394[e]	4	6307762	6308465
5.2	**CDO1049**	4	7497676	7500061
5.1	CDO344	4	8453360	8453651
5.1	CDO344	4	8457255	8457560

5.1	CDO344	4	8460278	8460619
5.1	CDO344	4	8464909	8465214
5.1	CDO344	4	8471117	8471457
5.1	CDO344	4	8474194	8474511
5.1	CDO344	4	8480368	8480693
5.1	CDO344	4	8483793	8484112
5.1	CDO344	4	8486603	8486922
5.1	CDO344	4	8493131	8493419
5.1	CDO344	4	8497047	8497331
5.1	CDO344	4	8502867	8503174
5.1	CDO344	4	8530596	8530922
5.1	CDO344	4	8539446	8539781
5.1	CDO344	4	8544334	8544651
5.1	CDO344	4	8547916	8548212
5.1-5.2	Ast563 (DY543444)	4	29522820	29523351
5.2	BCD1087	4	33816927	33817270
5.1-5.2	CDO989	4	36917718	36917857
5.1-5.2	CDO412	4	37681112	37681669
5.1	BCD1088	4	38888244	38888602
5.2	CDO127	4	46698455	46698943
6.1-6.2	**CDO1158**	1	27070854	27071168
6.1	**CDO686**	1	42666761	42667080
6.1-6.2	**CDO1158**	3	3998204	3998548
6.1-6.2	CDO534	3	5115466	5116010
6.1	Ast3163 (DY543439)	3	48111250	48114561
6.1-6.2	CDO497	3	49118762	49119005
6.2	Ast3199 (DY543537)	3	49305661	49306768
6.1-6.2	CDO1380	3	49359330	49359740
6.1	Ast370 (DY543428)	3	50900443	50900807
6.1-6.2	BCD1860	3	53533432	53533752
6.2	RZ273	3	54188671	54188821
6.2	CDO507	3	54190265	54190547
6.2	Ast3115[e]	3	55685645	55686559
6.1-6.2	Ast3155[e]	3	56863663	56864855
6.1	CDO516	3	57450800	57451023
6.1-6.2	**CDO686**	3	58868011	58868332
6.1	Ast5165 (DY543466)	4	45075939	45076791
7.1-7.2	Ast568 (DY543448)	1	12573959	12576486
7.1	**RZ508**	1	25302337	25302576
7.1	Ast554 (DY543415)	1	25880365	25881182
7.1-7.2	RZ682	1	26511824	26511978
7.1	CDO673	1	33021887	33022102
7.1-7.2	Ast5381 (DY543790)	1	34370529	34371141
7.1	**Ast5213** (DY543594)	1	35552937	35553492
7.1	Ast3213 (DY543567)	1	39989003	39990068
7.1-7.2	CDO17	1	40844693	40844982
7.1-7.2	Ast369 (DY543427)	1	41580006	41581370
7.2	Ast5115 (DY543497)	1	46751576	46751864
7.1	CDO545	1	49180863	49181176

7.1	RZ516	1	49336575	49336816
7.2	BCD93	1	50094042	50094446
7.1	**RZ508**	1	72954229	72954468
7.2	Ast5150 (DY543458)	2	6103348	6103548
7.1	**Ast5213** (DY543594)	2	24920787	24921343
7.1	**RZ508**	3	928721	928957
7.1	BCD349	3	11439298	11439995
7.1	BCD276	3	38400248	38401418
7.1-7.2	Ast5121 (DY543501)	3	39274997	39275975
7.1	CDO464	3	41043884	41046423
7.1	CDO595	3	42643624	42643830
7.2	BCD98	3	44128540	44129179
7.2	CDO99	3	44130090	44130609

[a] Creeping bentgrass linkage group (LG) where the marker was mapped.
[b] The markers in bold correspond to those that matched in more than one *B. distachyon* chromosome.
[c] *B. distachyon* chromosome where the marker was found after the BLASTN search.
[d] Location of the alignment between the marker and the *B. distachyon* chromosome (start and end correspond to base pair number).
[e] Sequence not available at NCBI

Table 5. Creeping bentgrass EST orthologs to *B. distachyon* chromosomes.

Agrostis	Bd chromosome location [a]		Bd	Duplications	
EST ID	Start	End	chromosome [b]	Within (#) [c]	Among (Bdch) [d]
DV860292	58122365	58122254	1		
DV860299	44145666	44145859	1		
DV860408	48045416	48045508	1		
DV860460	5767669	5767618	1		
DV860498	20235456	20235377	1		
DV860500	64837233	64838211	1		Bd3
DV860573	4696595	4696055	1	3	Bd4
DV860604	69418662	69418945	1		
DV860646	18654987	18654820	1		
DV860829	45677406	45677354	1		
DV860890	60661123	60661243	1		
DV860997	50443381	50443446	1		
DV861094	61951160	61951233	1		
DV861115	58331771	58331708	1		
DV861154	5190381	5190651	1		
DV861286	9087868	9087689	1		
DV861287	7148045	7148191	1		
DV861317	8149957	8150325	1		
DV861320	50081458	50081603	1		
DV861326	45677260	45677197	1		
DV861401	16199480	16198922	1	2	
DV861438	5190249	5190675	1		
DV861460	13812451	13812769	1		
DV861483	74281932	74281970	1		
DV861541	4094647	4094675	1		
DV861568	13812524	13812769	1		
DV861577	70726749	70726604	1		
DV861613	72499792	72499653	1		
DV861633	63754352	63754049	1		
DV861655	7976981	7976902	1		
DV861676	62732883	62732736	1		
DV861700	48423826	48424626	1		
DV861736	21906435	21906124	1		
DV861802	33222172	33221876	1		
DV861828	9773669	9773516	1		
DV861866	63094796	63094718	1		
DV861915	63607740	63607830	1		
DV862048	9019073	9019421	1		Bd2, 3, 4
DV862055	66614079	66614109	1		
DV862135	4109009	4109080	1		
DV862142	61924029	61923976	1	2	
DV862161	20665137	20664943	1		
DV862168	1827223	1827426	1		
DV862191	21169471	21169737	1		
DV862253	69507164	69506853	1		

DV862293	14550469	14550194	1		
DV862417	40736060	40735806	1	3	Bd2, 3, 4, 5
DV862436	62984506	62984239	1		
DV862455	8567980	8567530	1		
DV862491	15300725	15300920	1		
DV862560	65870991	65870793	1		
DV862581	27643540	27643518	1		
DV862694	17151824	17151560	1		
DV862801	50540315	50540420	1		
DV862836	7703418	7703152	1		
DV862864	6390655	6390506	1		
DV862946	14212470	14212518	1		
DV862967	28326959	28327251	1	3	Bd2
DV863053	12482891	12483277	1		
DV863161	4167967	4167607	1		Bd5
DV863251	3337557	3337358	1		
DV863289	41623175	41623058	1		
DV863339	54705284	54705750	1		
DV863383	50093974	50094303	1		
DV863417	68743290	68743522	1		
DV863428	62356412	62356589	1		
DV863533	8312570	8312298	1		
DV863568	57063707	57063877	1		
DV863585	27644182	27644136	1		
DV863610	68584591	68584504	1		
DV863711	41875484	41875567	1		
DV863776	68237345	68237305	1		
DV863894	66404574	66404632	1		
DV863905	7319394	7318933	1		
DV863972	63265235	63265356	1		
DV863977	70427108	70426975	1		
DV864001	31324229	31324410	1		
DV864040	1827016	1827060	1		
DV864053	20665860	20665690	1		
DV864078	61924016	61923976	1		
DV864090	65468808	65468574	1		
DV864141	66405253	66404970	1		
DV864224	67130227	67130126	1		
DV864253	70031121	70030905	1		
DV864353	15392991	15393057	1		
DV864466	62192267	62192146	1		
DV864484	65941385	65941133	1		
DV864629	58231019	58230905	1		
DV864659	61653588	61653529	1		
DV864674	24421769	24421956	1		
DV864721	50442967	50443035	1		
DV864795	67267510	67267295	1		
DV864836	68520537	68520642	1		
DV864856	32343727	32343608	1		

DV864873	64721655	64721282	1		
DV864877	53393692	53393817	1		
DV864895	33459406	33459594	1		
DV864903	34368864	34368949	1		
DV864919	20234724	20234779	1		
DV864929	5005786	5005835	1		
DV864935	64592884	64593196	1		
DV865001	32280141	32280519	1		
DV865003	68683540	68683948	1		
DV865043	28915490	28915411	1		
DV865075	1867227	1867175	1		
DV865098	6677073	6677035	1		
DV865127	46551540	46551366	1		
DV865133	7647707	7647819	1		
DV865195	8341195	8341289	1		
DV865392	10068223	10068158	1		Bd4
DV865471	62627778	62627626	1		
DV865532	62984068	62983804	1		
DV865533	15415853	15415740	1		
DV865669	54824816	54824718	1		
DV865674	54728480	54727963	1	2	Bd3
DV865696	64744478	64744433	1		
DV865701	23554411	23553534	1		Bd3, 5
DV865708	54728507	54727963	1	2	Bd3
DV865724	20997621	20996942	1		
DV865739	6796569	6797013	1		
DV865785	66593521	66593487	1		
DV865804	54728502	54727963	1	2	Bd3
DV865826	54728480	54727963	1	2	
DV865841	72877071	72876983	1		
DV865868	69647995	69647889	1		
DV865884	8115004	8115227	1		Bd2, 3, 4
DV865886	43151697	43151965	1		
DV865950	41875866	41875939	1		
DV865964	3828360	3828166	1		
DV865986	19136181	19135848	1		
DV866003	72138556	72138667	1		
DV866026	17183424	17183235	1		
DV866076	25391399	25391539	1		
DV866214	29785607	29785382	1		
DV866247	20109516	20109787	1		
DV866274	44341630	44341496	1		
DV866364	3217278	3217478	1		
DV866367	5802618	5802512	1		
DV866392	74078223	74078355	1		
DV866394	6819372	6819658	1		
DV866507	14019536	14019453	1		
DV866687	30385706	30385357	1		
DV866702	35760109	35759575	1	3	Bd3, 5

DV866776	31324341	31324410	1		
DV866824	65468866	65468574	1		
DV866921	8340282	8340361	1		Bd4
DV866922	44166696	44166921	1		
DV866929	2149343	2149313	1		
DV867049	6966390	6966466	1		
DV867162	10462049	10462189	1		
DV867180	68584555	68584504	1		
DV867204	69506655	69506731	1		
DV867236	16199534	16198927	1	2	
DV867322	55153808	55153276	1		
DV867327	62269705	62269766	1		
DV867350	66515083	66515016	1		
DV867402	18722397	18722044	1		
DV867410	44107086	44106533	1		
DV867431	3846565	3846703	1		
DV867599	22258140	22257826	1		
DV867624	3033162	3032946	1		
DV867642	50442963	50443044	1		
DV867821	22255772	22255637	1		
DV867864	65333302	65332849	1	2	Bd2, 4
DV867906	64232845	64232686	1		
DV867939	69319357	69319205	1		Bd2
DV867983	8332686	8332424	1		
DV868011	65871029	65870796	1		
DV868178	35676838	35677115	1		
DV868185	64232480	64232170	1		
DV868389	61315864	61315929	1		
DV868473	30732130	30732203	1		
DV868474	5005531	5005619	1		
DV868568	21392853	21393410	1		
DV868569	47050436	47050913	1		Bd2
DV868571	8567287	8567477	1		
DV868589	9106557	9106458	1		
DV868657	16184528	16184225	1		
DV868673	46551481	46551366	1		
DV868696	35767393	35767306	1		Bd3
DV860440	58828213	58828652	2		
DV860462	8130081	8130213	2		
DV860544	1942380	1942420	2		
DV860576	34715619	34715470	2		
DV860677	44943932	44943639	2		
DV860798	54950742	54950566	2		
DV860863	54885244	54884948	2		
DV861137	22735692	22736079	2		
DV861142	49899123	49899365	2		
DV861250	20686994	20687069	2		
DV861296	45586147	45586321	2		
DV861316	9053108	9053172	2		

DV861558	21810268	21810563	2		
DV861598	48682792	48682886	2		
DV861788	14654541	14654289	2		
DV861816	52560350	52560458	2		
DV861860	15745740	15745959	2		
DV861913	44385570	44385704	2		
DV862008	50520435	50520160	2		
DV862034	57071793	57071582	2		
DV862047	45333824	45333608	2		
DV862048	14366890	14366544	2		Bd1, 3, 4
DV862081	42711075	42710606	2		
DV862120	45267064	45267100	2		
DV862122	53025570	53025669	2		
DV862323	47926307	47926464	2		
DV862342	39007020	39007129	2		
DV862417	27605443	27605162	2		Bd1, 3, 4, 5
DV862428	48361001	48361277	2		
DV862475	25704451	25704594	2		
DV862658	34973349	34973455	2		
DV862727	42772559	42772621	2		
DV862859	27937279	27937380	2		
DV862967	34823412	34823373	2		Bd1
DV862973	42699139	42698868	2		Bd4, 5
DV862975	31967633	31967706	2		
DV863075	10775828	10776170	2		
DV863175	79191	79132	2		
DV863230	54913122	54913017	2		
DV863397	4131594	4131919	2		
DV863415	48702669	48702487	2		
DV863483	9426644	9426437	2		
DV863513	51187722	51187665	2		
DV863731	13885525	13885704	2		
DV863768	23277511	23277543	2		
DV863834	52751022	52751229	2		
DV863912	41541235	41541312	2		
DV864036	14654768	14654655	2		
DV864079	4874407	4873819	2		
DV864101	56324416	56324554	2		
DV864179	21525833	21526263	2		
DV864372	25387498	25387430	2		
DV864419	26128710	26128637	2		
DV864452	34559406	34559520	2		
DV864519	79103	78833	2		
DV864523	36805299	36805225	2		
DV864592	5535433	5535613	2	9	Bd3, 4, 5
DV864635	54698355	54698422	2		
DV864651	53521548	53521800	2		
DV864662	8647615	8647514	2		
DV864717	56324456	56324554	2		

DV864728	52517704	52517931	2		
DV864805	22829085	22828968	2		
DV864807	50017778	50017805	2		
DV864815	52389674	52389726	2		
DV864816	35174238	35174298	2		
DV864916	17396311	17396249	2		
DV864951	46311067	46310901	2		
DV864983	37775200	37775080	2		
DV865067	15854897	15854979	2		Bd5
DV865205	52015374	52015014	2	2	
DV865327	23489395	23489561	2		
DV865346	7157796	7157879	2		
DV865350	20681751	20681892	2	2	
DV865377	18485150	18485260	2		
DV865535	17737977	17737908	2		
DV865704	18328549	18328731	2		
DV865745	17737309	17737175	2		
DV865828	14730938	14730897	2		
DV865858	45333820	45333608	2		
DV865884	46083720	46083505	2	4	Bd1, 3, 4
DV865915	55881898	55881959	2		
DV865926	48854619	48854284	2		
DV866059	47349902	47349872	2		
DV866122	56980647	56980899	2		
DV866138	13931114	13931197	2		
DV866190	34514569	34514488	2		
DV866201	58846528	58846752	2		
DV866217	34715333	34715190	2		
DV866231	56169106	56169003	2		
DV866278	819649	820190	2	2	
DV866368	48359974	48360036	2		
DV866408	35945581	35945804	2		
DV866416	47445494	47445763	2		
DV866460	6723355	6723285	2		
DV866463	25704620	25704893	2		
DV866611	51077282	51077167	2		
DV866712	48360330	48360423	2		
DV866724	53137067	53137472	2		
DV866729	40629580	40630011	2		
DV866808	20797998	20797882	2		
DV866845	18329267	18329053	2		
DV866876	35296115	35296479	2		
DV867055	10495784	10495399	2		
DV867425	45787907	45787873	2		
DV867712	11494377	11493479	2	2	Bd3
DV867831	25508283	25508196	2		
DV867864	20792554	20792906	2	2	Bd1, 4
DV867908	52480863	52480946	2		
DV867939	7218301	7218453	2		

DV868191	21033688	21033740	2		
DV868386	34339496	34339419	2		
DV868415	49834388	49834740	2		
DV868462	42039673	42039736	2		
DV868512	46502209	46502009	2		
DV868569	15977039	15976552	2	2	Bd1
DV868618	52190423	52190047	2		
DV868699	18081321	18081370	2		
DV868717	58846361	58846404	2		
DV860413	57592315	57592604	3		
DV860467	41827196	41826633	3		
DV860484	14660906	14660670	3		
DV860500	26475457	26476433	3		Bd1
DV860566	43602264	43602433	3		
DV860613	9538980	9539361	3		
DV860681	55140095	55140373	3		
DV860756	24423176	24423053	3		
DV860895	55140137	55140373	3		
DV861045	55820244	55819898	3		
DV861113	36891442	36891133	3		
DV861170	5230478	5230712	3		
DV861212	28301395	28301269	3		
DV861458	49035386	49035420	3		
DV861464	35237938	35238426	3		
DV861493	45748962	45748911	3		
DV861509	20773241	20772793	3		
DV861526	4234010	4234423	3		
DV861537	53307319	53307496	3		
DV861784	5230712	5230469	3		
DV861786	6822935	6822678	3		
DV861842	33515698	33515503	3		
DV861859	18005976	18005900	3		
DV861876	54190217	54190548	3		
DV861883	3589294	3589356	3		
DV862048	44114069	44114417	3		Bd1, 2, 4
DV862087	51607353	51607297	3		
DV862175	52059922	52060115	3		
DV862209	27093561	27093458	3		Bd4, 5
DV862232	47189109	47189388	3		
DV862248	46634216	46634359	3		
DV862254	11310459	11309975	3		
DV862334	6584285	6584137	3		
DV862417	31678620	31678309	3	3	Bd1, 2, 4, 5
DV862562	53982648	53982619	3		
DV862591	34216918	34217129	3		
DV862624	2683106	2682823	3		
DV862689	41216693	41216912	3		
DV862703	54139041	54138920	3		
DV862767	2116200	2116120	3		

DV862948	29195056	29195153	3		
DV863002	53349136	53349260	3		
DV863101	54190304	54190548	3		
DV863192	54190241	54190546	3		
DV863431	36345148	36345300	3		
DV863446	13270142	13269770	3		
DV863555	55140258	55140373	3		
DV863594	37368021	37368100	3		
DV863664	53348929	53349260	3		
DV863673	49306759	49306504	3		
DV863873	17661430	17661745	3		
DV864207	14785676	14785599	3		
DV864229	58563247	58562887	3		
DV864230	11902479	11902181	3		
DV864327	2601565	2601748	3		
DV864343	337028	336766	3		
DV864382	49951702	49952106	3		
DV864458	58898957	58899115	3		
DV864525	46632856	46633079	3		
DV864540	21374341	21374373	3		
DV864553	14438066	14437928	3		
DV864557	47725037	47725109	3		
DV864561	41861655	41861108	3	3	
DV864592	9405379	9405482	3	9	Bd2, 4, 5
DV864600	52766934	52766564	3		
DV864630	24456747	24456584	3		
DV864680	1832061	1831933	3		
DV864793	56413098	56413248	3		
DV864818	50076027	50075975	3		
DV864850	52767894	52767791	3		
DV864994	44093484	44093418	3		
DV865022	41334364	41334141	3		
DV865074	15179324	15179068	3		
DV865130	55140018	55140373	3		
DV865247	6737570	6737609	3		
DV865446	3799323	3799259	3		
DV865545	52611933	52612227	3		
DV865674	28296881	28296365	3		Bd3
DV865689	38984160	38984084	3		
DV865701	59882148	59881463	3		Bd1, 5
DV865707	7587848	7588313	3		
DV865708	28296908	28296365	3		Bd1
DV865723	43626765	43626818	3		
DV865804	28296903	28296365	3		Bd1
DV865826	28296881	28296365	3		
DV865884	1260196	1260104	3	6	Bd1, 2, 4
DV866009	49035313	49035420	3	2	
DV866029	48566785	48566680	3		
DV866042	16249343	16249408	3		

DV866055	46867731	46867839	3		
DV866058	53308274	53308036	3		
DV866079	41216070	41216163	3		
DV866085	54190311	54190546	3		
DV866097	48293931	48293961	3		
DV866118	42077347	42077437	3		
DV866134	53986653	53986595	3		
DV866137	53212701	53212662	3		
DV866171	21962376	21962519	3		
DV866329	2116161	2116116	3		
DV866488	39621971	39621881	3		
DV866557	55686020	55686086	3		
DV866702	26473816	26473283	3	2	Bd1, 5
DV866764	36079096	36079123	3		
DV866879	55473254	55473386	3		
DV866906	50602678	50602552	3		
DV866945	5325172	5324854	3		
DV866947	19004342	19004259	3		
DV866994	45749061	45748911	3		
DV867088	27092988	27093144	3	2	Bd4, 5
DV867365	32196984	32196364	3		
DV867452	50072981	50073043	3		
DV867607	4791079	4790646	3		
DV867630	39010016	39009952	3		
DV867631	57693466	57693525	3		
DV867712	28306471	28307335	3		Bd2
DV867720	54698832	54698904	3		
DV867867	32000393	32000422	3		
DV867874	53307259	53307496	3		
DV867975	42609386	42609633	3		
DV868003	34849296	34849437	3		
DV868026	12516856	12516792	3		
DV868235	48294120	48294174	3		
DV868245	27092986	27093371	3		Bd4, 5
DV868314	995357	995185	3		
DV868382	34231467	34231259	3		
DV868445	53307889	53307984	3		
DV868526	38400870	38400966	3		
DV868603	44238425	44238525	3		Bd4
DV868608	35808972	35809225	3		
DV868655	36451259	36450614	3		
DV868696	33554205	33554502	3		Bd1
DV860562	5998061	5997972	4		
DV860573	12827393	12827931	4		Bd1
DV860617	23335470	23335842	4		
DV860816	6943097	6943307	4		
DV861225	2860959	2860524	4		
DV861247	41342398	41342428	4		
DV861347	9381465	9381570	4		

DV861636	4905440	4905332	4		
DV861734	7477414	7477380	4		
DV861753	32664644	32664666	4		
DV862048	42403184	42403529	4		Bd1, 2, 3
DV862209	7941422	7941479	4		Bd3, 5
DV862417	41377627	41377317	4		Bd1, 2, 3, 5
DV862668	3061596	3061518	4		
DV862928	35863629	35863411	4		
DV862938	40525915	40526007	4		
DV862973	3472143	3472495	4	16	Bd2, 5
DV863232	2311169	2311316	4	4	Bd5
DV863635	13767568	13767051	4	3	
DV864085	7547767	7547646	4	2	
DV864184	8423213	8423511	4		
DV864355	37802805	37802859	4		
DV864369	33709656	33709864	4		
DV864424	37613848	37613993	4		
DV864475	26439698	26440071	4		
DV864528	200144	199930	4		
DV864592	8131489	8131669	4	8	Bd2, 3, 5
DV864830	33887378	33887554	4		
DV864889	33709656	33709864	4		
DV864966	32725916	32725872	4		
DV865096	45035187	45035264	4		
DV865392	757131	757066	4		Bd1
DV865666	5569885	5570111	4		
DV865884	3951132	3951040	4	5	Bd1, 2, 3
DV865890	8422997	8423093	4		
DV865966	31049668	31049571	4		
DV866068	45035166	45035264	4		
DV866159	24618071	24617821	4		
DV866573	8423334	8423511	4		
DV866645	41721218	41721185	4	2	
DV866653	27015231	27015379	4	2	
DV866717	1071167	1071376	4		
DV866731	36215370	36215424	4		
DV866847	16568599	16568411	4		
DV866866	32295119	32294863	4		
DV866921	17458499	17458445	4		Bd1
DV866965	43773471	43773595	4		
DV867026	30355538	30355387	4		
DV867088	7942029	7941877	4	2	Bd3, 5
DV867116	37910205	37910075	4		
DV867418	3357310	3357338	4		
DV867542	36215197	36215424	4		
DV867756	35744568	35744514	4		
DV867864	3420284	3420648	4		Bd1, 2
DV867978	41146228	41146272	4		
DV868017	29882056	29881815	4	2	

DV868027	30354683	30355233	4		
DV868078	41808838	41808767	4		
DV868245	7546855	7547238	4		Bd3, 5
DV868253	7497078	7496803	4		
DV868381	8423364	8423464	4		
DV868411	8423333	8423512	4		
DV868417	23699443	23699364	4		
DV868603	43417370	43417462	4		Bd3
DV868616	39121245	39121177	4		
DV868639	17457309	17457234	4		
DV860321	556999	556867	5		
DV862105	26152340	26152471	5		
DV862209	5280265	5280384	5		Bd3, 4
DV862417	2371119	2371408	5		Bd1, 2, 3, 4
DV862454	9879775	9879416	5		
DV862599	13475166	13475477	5		
DV862716	22418989	22419077	5		
DV862906	9617332	9617129	5		
DV862933	22419478	22419344	5		
DV862973	323610	323650	5	6	Bd2, 4
DV863161	18173955	18173595	5		Bd1
DV863232	1636986	1637171	5	5	Bd4
DV863372	20009427	20009345	5		
DV863522	15228513	15228449	5		
DV863707	25383635	25383680	5		
DV863732	26268894	26268812	5		
DV863777	17060599	17060864	5		
DV863851	19043205	19042880	5		
DV863880	9812091	9811993	5		
DV863987	19757998	19758208	5		
DV864085	5280260	5280384	5		
DV864145	9810291	9810267	5		
DV864301	6768918	6768952	5		
DV864385	3127656	3127514	5		
DV864567	9618288	9618248	5		
DV864592	2865773	2865950	5	5	Bd2, 3, 4
DV864607	17666190	17666155	5		
DV864930	17418268	17418535	5		
DV865067	23463062	23462980	5		Bd2
DV865147	21116726	21116851	5		
DV865243	19226028	19225990	5		
DV865579	22418954	22419077	5		
DV865701	5536	4659	5	30	Bd1, 3
DV865833	19197894	19197970	5		
DV865909	16097438	16097918	5		
DV866106	9704864	9704790	5		
DV866389	20009378	20009345	5		
DV866651	14833840	14833799	5		
DV866689	12974472	12974588	5		

DV866702	25646469	25647003	5	Bd1, 3
DV867088	5281337	5281181	5	Bd3, 4
DV867307	22015019	22015084	5	
DV867384	4345446	4345302	5	
DV867516	16032678	16032634	5	
DV867620	15704364	15704614	5	
DV867663	10410936	10411180	5	
DV868245	5281340	5280955	5	Bd3, 4
DV868429	22094662	22094785	5	
DV868456	25681656	25681962	5	

[a] Location of the alignment between the marker and the *B. distachyon* chromosome (start and end correspond to base pair number).
[b] *B. distachyon* chromosome where the creeping bentgrass EST was found as ortholog.
[c] Number of multiple matches of Creeping bentgrass EST orthologs within one *B. distachyon* chromosome.
[d] Additional *B. distachyon* chromosome where the creeping bentgrass EST was found as ortholog.

Table 6. Disease resistance and defense response genes identified in creeping bentgrass ESTs and their homologous peptides in *B. distachyon*.

Agrostis EST ID	*Brachypodium* locus ID	*Brachypodium* chromosome	Protein family
DV863491	Bradi1g72430	1	Leucine rich repeat
DV864848	Bradi1g05950	1	Protein kinase domain
DV861655	Bradi1g10970	1	Protein kinase domain
DV867398	Bradi1g58400	1	Protein kinase domain
DV861137	Bradi2g24930	2	Protein kinase domain
DV866278	Bradi2g01320	2	Protein kinase domain
DV867860	Bradi2g06060	2	Protein kinase domain
DV865535	Bradi2g20200	2	Protein kinase domain
DV863115	Bradi3g47600	3	Protein kinase domain
DV860937	Bradi4g26410	4	Protein kinase domain
DV862787	Bradi4g33110	4	Protein kinase domain
DV865579	Bradi5g19430	5	Protein kinase domain
DV863880	Bradi5g07360	5	Protein kinase domain

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